Guidance for the risk assessment of the presence at low level of genetically modified plant material in imported food and feed under Regulation (EC) No 1829/2003

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SCIENTIFIC OPINION

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Scientific Opinion on application EFSA-GMO-BE-2013-118 for authorisation of genetically modified maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 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),
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Abstract

In this opinion, the GMO Panel assessed the five-event stack maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and its 25 subcombinations, independently of their origin. The GMO Panel has previously assessed the five single events combined to produce this five-event stack maize and 11 subcombinations of these events and did not identify safety concerns. No new data on the single events or their previously assessed subcombinations, leading to modification of the original conclusions were identified. The combination of the single events and of the newly expressed proteins in the five-event stack maize did not give rise to issues – based on the molecular, agronomic/phenotypic or compositional characteristics – regarding food and feed safety and nutrition. Considering the scope of this application, the known biological function of the newly expressed proteins and the data available for the five-event stack maize and its previously assessed maize subcombinations, the GMO Panel considered that different combinations of the single events would not raise environmental concerns. The GMO Panel concludes that the five-event stack maize is as safe and as nutritious as the non-genetically modified (GM) comparator and the tested non-GM reference varieties in the context of its scope. For the 14 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 combinations would not raise safety concerns. These maize subcombinations are therefore expected to be as safe as the single events, the previously assessed subcombinations and maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122. Since the post-market environmental monitoring plan for the five-event stack maize does not include any provisions for the 14 maize subcombinations not previously assessed, the GMO Panel recommended the applicant to revise the plan accordingly.

Keywords: GMO, maize, MON 87427 × MON 89034 × 1507 × MON 88017 × 59122, herbicide tolerance, insect resistance

Requestor: Competent Authority of Belgium

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Summary

Following the submission of application EFSA-GMO-BE-2013-118 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 (referred to hereafter as GMO Panel) was asked to deliver a Scientific Opinion on the safety of genetically modified glufosinate-ammonium- and glyphosate-tolerant and insect resistant maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 (referred to hereafter as 'five-event stack maize') and its subcombinations independently of their origin, according to the Commission Implementing Regulation (EU) No 503/2013 (referred to hereafter as 'subcombinations'). The scope of application EFSA-GMO-BE-2013-118 is for the placing on the market of maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 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 four of the events present in the five-event stack maize. The safety of subcombinations occurring as segregating progeny in the harvested grains of maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 is evaluated in the context of the assessment of the five-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 five-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 five-event stack maize, a four-event and two two-event stack subcombinations, the scientific comments submitted by the Member States and the relevant scientific literature. The five-event stack maize was produced by conventional crossing to combine five 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; 1507 expressing the Cry1F protein which confers protection against specific lepidopteran pests and phosphinothricin acetyl transferase (PAT) protein for tolerance to glufosinate-containing herbicides; MON 88017 expressing the Cry3Bb1 protein to confer protection against coleopteran pests belonging to the genus *Diabrotica*, such as the western corn rootworm (*Diabrotica virgifera virgifera*) and CP4 EPSPS protein for tolerance to glyphosate-containing herbicides; and 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-containing herbicides.

The GMO Panel evaluated the five-event stack maize and its subcombinations with reference to the scope and appropriate principles described in its guidelines for the risk assessment of genetically modified (GM) plants and derived food and feed, the environmental risk assessment of GM plants and the post-market environmental monitoring of GM plants. The 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-2013-118, previous assessments of the five single maize events (MON 87427, MON 89034, 1507, MON 88017 and 59122), the four-event stack maize MON 89034 × 1507 × MON 88017 × 59122 and all its subcombinations and of the two two-event stack maize events (1507 × 59122 and MON 89034 × MON 88017) provided a basis to evaluate the five-event stack maize and all its subcombinations. Maize MON 87427, MON 89034, 1507, MON 88017, 59122, 1507 × 59122, MON 89034 × MON 88017 and MON 89034 × 1507 × MON 88017 × 59122 (and all its subcombinations) were previously assessed by the GMO Panel and no concerns on their safety were identified. No safety issue concerning the five 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 five-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 × 1507 × MON 88017 × 59122 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins are similar in the five-event stack maize and in the single events except for the expected difference for the CP4 EPSPS and PAT protein levels resulting from the combination of MON 87427 and MON 88017 single events, both producing CP4 EPSPS protein, and 1507 and 59122 single events, both producing PAT protein in the five-event stack. No indications of interactions that may affect the integrity of the events and the levels of the newly expressed proteins in this five-event stack maize were identified.

No relevant differences between maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 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, except for a decrease of thiamin in the GM maize.

Based on the molecular, agronomic, phenotypic or compositional characteristics, the combination of maize events MON 87427, MON 89034, 1507, MON 88017 and 59122 in the five-event stack maize did not give rise to issues regarding food and feed safety and nutrition. The nutritional assessment identified no concerns related to the decrease of thiamin. The combination of the newly expressed proteins in the five-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 this five-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 this five-event stack maize.

The GMO Panel concludes that the five-event stack maize is as safe and as nutritious as the non-GM comparator and 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 maize MON 89034 × MON 88017, MON 89034 × MON 88017, MON 89034 × 1507 × MON 88017 × 59122 and its subcombinations, 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 the 14 maize subcombinations included in the scope of EFSA-GMO-BE-2013-118 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, 1507, MON 88017 and 59122 would not raise safety concerns. These maize subcombinations are therefore expected to be as safe as the single events, the previously assessed maize subcombinations and the five-event stack maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122.

Given the absence of safety concerns identified on food and feed derived from maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and MON 89034 × MON 88017, MON 89034 × MON 88017, MON 89034 × 1507 × MON 88017 × 59122 and its subcombinations, 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 five-event stack maize does not include any provisions for the 14 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 26 November 2013, the European Food Safety Authority (EFSA) received from the Competent Authority of Belgium application EFSA-GMO-BE-2013-118, for authorisation of genetically modified (GM) insect-resistant and herbicide-tolerant maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 (referred to hereafter as five-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-2013-118 presented here is for the placing on the market of five-event stack maize and all its subcombinations independently of their origin, for food and feed uses, import and processing (see Table 1).

After receiving application EFSA-GMO-BE-2013-118 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.[2] 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 17 January 2014 and received it on 17 February 2014. On 10 March 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 13 March 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[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 (until 11 September 2015[4]) to make their opinion known.

The GMO Panel carried out the scientific risk assessment of the five-event stack maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and subcombinations (referred to as 'subcombinations independently of their origin' according to the Commission Implementing Regulation (EU) No 503/2013[5]). The GMO Panel requested additional information from the applicant on 11 June 2015, 30 July 2015, 26 November 2015, 11 December 2015, 8 March 2016, 28 June 2016, 4 August 2016, 11 August 2016, 25 October 2016, 11 April 2017 and 16 May 2017. The applicant provided the requested information on 6 July 2015, 2 October 2015, 18 December 2015, 13 June 2016, 11 July 2016, 29 July 2016, 1 September 2016, 15 September 2016, 24 November 2016, 25 November 2016, 6 June 2017 and 20 June 2017, respectively. The applicant provided additional information spontaneously on 25 November 2016 and 13 December 2016.

In the context of contract 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).

[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, L268, 1–23.

[2] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2013-00926

[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. OJ L 106, 12.3.2001, p. 1–38.

[4] The Member States' commenting period of application EFSA-GMO-BE-2013-118 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).

[5] 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 L157, 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 × 1507 × MON 88017 × 59122 and all maize 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-2013-118 presented here is for the placing on the market of glyphosate- and glufosinate-tolerant and insect-resistant maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and all its 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 genetically modified organisms (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-2013-118, 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 × 1507 × MON 88017 × 59122 and all its subcombinations independently of their origin (see 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 and were taken into consideration during the scientific risk assessment.

3. Assessment

3.1. Introduction

Application EFSA-GMO-BE-2013-118 covers the five-event stack maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122[6] and all its subcombinations 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 up to four of the events present in the five-event stack maize.

The safety of subcombinations occurring as segregating progeny in the harvested grains of maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 is evaluated in the context of the assessment of the five-event stack maize in Section 3.3 of the present GMO Panel Scientific Opinion.

'Subcombination' also covers combinations of up to four of the five events MON 87427, MON 89034, 1507, MON 88017 or 59122 that have either been, or could be produced by conventional crossing through targeted breeding approaches (EFSA GMO Panel, 2011a). These are maize stacks

[6] Unique identifier: MON-87427-7 × MON-89Ø34-3 × DAS-Ø15Ø7-1 × MON-88Ø17-3 × DAS-59122-7.

that can be bred, produced and marketed independently of the five-event stack maize. These stacks are risk assessed in the Section 3.4 of this GMO Panel Scientific Opinion.

The five-event stack maize was produced by conventional crossing to combine five single maize events: MON 87427 (expressing the 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein); MON 89034 (expressing the Cry1A.105 and Cry2Ab2 proteins); 1507 (expressing the Cry1F and phosphinothricin acetyl transferase (PAT) proteins); MON 88017 (expressing the Cry3Bb1 and CP4 EPSPS proteins); 59122 (expressing the Cry34Ab1, Cry35Ab1 and PAT proteins).

Table 1: Stacked maize events covered by the scope of application EFSA-GMO-BE-2013-118

Degree of stacking	Events
Five-event stack maize	MON 87427 × MON 89034 × 1507 × MON 88017 × 59122
Four-event stack maize	MON 89034 × 1507 × MON 88017 × 59122
	MON 87427 × 1507 × MON 88017 × 59122
	MON 87427 × MON 89034 × 1507 × 59122
	MON 87427 × MON 89034 × 1507 × MON 88017
	MON 87427 × MON 89034 × MON 88017 × 59122
Three-event stack maize	1507 × MON 88017 × 59122
	MON 89034 × 1507 × 59122
	MON 89034 × 1507 × MON 88017
	MON 89034 × MON 88017 × 59122
	MON 87427 × 1507 × 59122
	MON 87427 × 1507 × MON 88017
	MON 87427 × MON 88017 × 59122
	MON 87427 × MON 89034 × 1507
	MON 87427 × MON 89034 × 59122 MON 87427 × MON 89034 × MON 88017
Two-event stack maize	1507 × 59122
	MON 88017 × 59122
	MON 89034 × 1507
	MON 89034 × 59122
	MON 89034 × MON 88017
	1507 × MON 88017
	MON 87427 × 1507
	MON 87427 × 59122
	MON 87427 × MON 88017
	MON 87427 × MON 89034

Herbicidal tolerance traits are achieved by the expression of CP4 EPSPS protein from *Agrobacterium* sp. strain CP4 and PAT protein from *Streptomyces viridochromogenes*. Insecticidal resistance traits are achieved by the expression of the Cry1A.105, Cry2Ab2 and Cry1F proteins from *Bacillus thuringiensis* subsp. *kurstaki* and subsp. *aizawai*, which confer protection against specific lepidopteran pests, such as the European corn borer (*Ostrinia nubilalis*) and by the expression of the Cry3Bb1, Cry34Ab1 and Cry35Ab1 from *B. thuringiensis* (strain PS149B1), which confers protection against specific coleopteran pests belonging to the genus *Diabrotica*, such as the Western corn rootworm (*Diabrotica virgifera virgifera*).

All five single maize events, two-event stacks 1507 × 59122 and MON 89034 × MON 88017 as well as the four-event stack maize MON 89034 × 1507 × MON 88017 × 59122 and all its subcombinations independently of their origin have been previously assessed by the GMO Panel (see Table 2), and no safety concerns were identified.

Table 2: Single maize events and subcombinations of maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 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)
1507	C/NL/00/10	EFSA (2004)
	C/ES/01/01,2001/18/EC	EFSA (2005a)
	EFSA-GMO-NL-02	EFSA (2005b)
	EFSA-GMO-RX-1507	EFSA (2009)
	EFSA-GMO-RX-001	EFSA GMO Panel (2017a)
MON 88017	EFSA-GMO-CZ-2007-27	EFSA GMO Panel (2009a)
59122	EFSA-GMO-NL-2005-12	EFSA (2007)
	EFSA-GMO-NL-2005-23	EFSA GMO Panel (2013a,b)
	EFSA-GMO-RX-003	EFSA GMO Panel (2017b)
1507 × 59122	EFSA-GMO-NL-2005-15	EFSA GMO Panel (2009b)
MON 89034 × MON 88017	EFSA-GMO-NL-2007-39	EFSA GMO Panel (2010b)
MON 88017 × 59122	EFSA-GMO-CZ-2008-62	EFSA GMO Panel (2010c)
1507 × MON 88017	EFSA-GMO-CZ-2008-62	EFSA GMO Panel (2010c)
MON 89034 × 1507	EFSA-GMO-CZ-2008-62	EFSA GMO Panel (2010c)
MON 89034 × 59122	EFSA-GMO-CZ-2008-62	EFSA GMO Panel (2010c)
1507 × MON 88017 × 59122	EFSA-GMO-CZ-2008-62	EFSA GMO Panel (2010c)
MON 89034 × 1507 × 59122	EFSA-GMO-CZ-2008-62	EFSA GMO Panel (2010c)
MON 89034 × 1507 × MON 88017	EFSA-GMO-CZ-2008-62	EFSA GMO Panel (2010c)
MON 89034 × MON 88017 × 59122	EFSA-GMO-CZ-2008-62	EFSA GMO Panel (2010c)
MON 89034 × 1507 × MON 88017 × 59122	EFSA-GMO-CZ-2008-62	EFSA GMO Panel (2010c)

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 (see Table 2), no safety issue concerning the five single events has been reported by the applicant. The applicant clarified that the 59122 maize sequence reported for the five-event stack maize was the sequence submitted in the original application EFSA-GMO-NL-2005-12 (EFSA, 2007), but corrected for sequencing errors affecting three single nucleotides[7] (EFSA GMO Panel, 2016). In addition, the applicant clarified that the 1507 maize sequence reported for the five-event stack maize contained two nucleotide changes in the insert sequence compared to the original, corrected 1507 maize sequence (EFSA GMO Panel, 2017a). Analysis of the new sequencing data and the bioinformatic analyses performed on the new sequence did not give rise to specific safety issues.[8,9]

Updated bioinformatic analyses on the junction regions for events MON 87427, MON 89034, 1507, MON 88017 and 59122 confirmed that no known endogenous genes were disrupted by any of the inserts.[7,8,10] Updated bioinformatic analyses of the amino acid sequence of the newly expressed Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS proteins revealed no significant similarities to toxins and allergens.[7,8,10] In addition, updated bioinformatic analyses of the

[7] Additional information: 15/9/2016 and 24/11/2016.
[8] Additional information: 25/11/2016.
[9] Additional information: 20/6/2017.
[10] Additional information: 29/7/2016.

newly created Open Reading Frames (ORFs) within the inserts and at their junctions for events MON 87427, MON 89034, 1507, MON 88017 and 59122 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 sliding window approach. 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 to the Cry2Ab2 ORF and does not contain any in-frame translational start codons (ATG).[11] 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 × 1507 × MON 88017 × 59122 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 for events MON 87427, MON 89034, 1507, MON 88017 and 59122 to microbial DNA. The likelihood and potential consequences of plant-to-bacteria gene transfer are described in Section 3.3.4.

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 five-event stack maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122

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[12]

Maize events MON 87427, MON 89034, 1507, MON 88017 and 59122 were combined by conventional crossing to produce event MON 87427 × MON 89034 × 1507 × MON 88017 × 59122. The structure of the inserts introduced into maize MON 87427, MON 89034, 1507, MON 88017 and 59122 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 × 1507 × MON 88017 × 59122 are summarised in Table 4.

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

Table 3: Genetic elements in the expression cassettes of the events stacked in maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122

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* (*Bacillus thuringiensis*)	*hsp17* (*Triticum* sp.)
	35S (FMV)	–	CTP (*Z. mays*)	*cry2Ab2* (*B. thuringiensis*)	*nos* (*A. tumefaciens*)
1507	*ubi*ZM1 (*Zea mays*)	–	–	*cry1F* (*B. thurigiensis*)	ORF25PolyA (*A. tumefaciens*)
	35S (CaMV)	–	–	*pat* (*Streptomyces viridochromogenes*)	35S (CaMV)

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

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
MON 88017	*act1* (*Oryza sativa*)	–	CTP2 (*A. thaliana*)	CP4 *epsps** (*Agrobacterium* sp.)	*nos* (*A. tumefaciens*)
	35S (CaMV)	CAB (*Triticum* sp.)	–	*Cry3Bb1* (*B. thuringiensis*)	*hsp17* (*Triticum* sp.)
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)

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

Table 4: Characteristics and intended effects of the events stacked in maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122

Event	Protein	Donor organism and biological function	Intended effects in GM plant
MON 87427	CP4 EPSPS	Donor organism: *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)	The expression of the bacterial CP4 EPSPS in event MON 87427 confers tolerance to glyphosate-containing herbicides as it has lower affinity towards glyphosate than the plant endogenous enzyme
MON 89034	Cry1A.105	Donor organism: *B. 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
	Cry2Ab2	Donor organism: *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 the Cry2Ab2 protein, which is toxic to certain lepidopteran larvae
1507	Cry1F	Donor organism: *Bacillus thuringiensis* subsp. *aizawai*. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes	Event 1507 expresses a truncated *cry1F* gene which was modified to enhance expression in plants. The amino acid sequence of the truncated part was not modified except for a single amino acid substitution, phenylalanine to leucine at position 604. Cry1F is a protein toxic to certain lepidopteran larvae
	PAT	Donor organism: *Streptomyces viridochromogenes* strain Tü494 Phosphinothricin-acetyl-transferase (PAT) enzyme confers resistance to the antibiotic bialaphos (Wohlleben et al., 1988)	Event 1507 expressed PAT, which acetylates L-glufosinate-ammonium and thereby confers tolerance to glufosinate ammonium-containing herbicides

Event	Protein	Donor organism and biological function	Intended effects in GM plant
MON 88017	CP4 EPSPS	Donor organism: *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 expression of the bacterial CP4 EPSPS in event MON 88017 confers tolerance to glyphosate-containing herbicides as it has lower affinity towards glyphosate than the plant endogenous enzyme
	Cry3Bb1	Donor organism: *B. thuringiensis* subsp. *kumamotoensis*. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes (Ellis et al., 2002)	Event MON 88017 expresses the Cry3Bb1 protein, which is toxic to certain lepidopteran larvae
59122	Cry34Ab1	Donor organism: *B. thuringiensis* strain PS149B1. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes	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	Donor organism: *B. thuringiensis* strain PS149B1. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes	Event 59122 expresses a *cry35Ab1* gene which was modified for enhanced expression in plants. The amino acid sequence was not modified. The Cry35Ab1 protein is toxic to certain coleopteran larvae feeding on maize
	PAT	Donor organism: *Streptomyces viridochromogenes.* Phosphinothricin-acetyl-transferase (PAT) enzyme confers resistance to the antibiotic bialaphos	Event 59122 expressed PAT, which acetylates L-glufosinate-ammonium and thereby confers tolerance to glufosinate ammonium-containing herbicides

3.3.1.2. Integrity of the events in the five-event stack maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122[10]

The genetic stability of the inserted DNA over multiple generations in single maize events MON 87427, MON 89034, 1507, MON 88017 and 59122 was demonstrated previously (see Table 2). Integrity of these events in maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 was demonstrated by Southern analyses.

3.3.1.3. Information on the expression of the inserts[13]

Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS protein levels were analysed by enzyme-linked immunosorbent assay (ELISA) in material harvested from replicated field trials at five locations in the US in the 2010 growing season.

Samples analysed included leaf (V2–V4), root (V2–V4 and R5), whole plant (V10–V12), forage (R5), pollen (pollination) and grain (R6) both those treated and not treated with glyphosate and/or glufosinate. 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 × 1507 × MON 88017 × 59122 (the highest mean values, regardless the treatment) are summarised in Table 5.

[13] Dossier: Part II – Section A2.2.3, study MSL0024204; additional information: 18/12/2015.

Table 5: Means, standard deviations and ranges of protein levels (μg/g dry weight) in grains (n = 19 or n = 20) and forage (n = 20) from maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122

Protein	Tissue/developmental stage	
	Grain/R6	Forage/R5
CP4 EPSPS	7.6[(a)] ± 1.1[(b)] (5.8–11)[(c)]	190 ± 50 (100–270)
Cry1A.105	8.1 ± 2.4 (6.0–14)	32 ± 8.6 (16–45)
Cry2Ab2	1.8 ± 0.40 (1.2–2.8)	39 ± 10.0 (17–58)
Cry1F	2.0 ± 0.33 (1.6–2.8)	5.0 ± 1.2 (3.1–7.3)
PAT	< LOD[(d)]	0.85 ± 0.30 (0.17–1.1)
Cry3Bb1	7.8 ± 1.1 (6.1–9.6)	50 ± 11 (30–69)
Cry34Ab1	35 ± 4.3 (29–42)	99 ± 23 (66–160)
Cry35Ab1	0.55 ± 0.11 (0.39–0.80)	12 ± 3.2 (6.9–18)

(a): Mean.
(b): Standard deviation.
(c): Range.
(d): LOD: limit of detection.

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

The levels of all the newly expressed proteins in the five-event stack maize and the corresponding singles were similar in all tissues except for the expected difference for the CP4 EPSPS and PAT protein levels resulting from the combination of MON 87427 and MON 88017 single events, both producing CP4 EPSPS protein, and 1507 and 59122 single events, both producing PAT protein in the five-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 × 1507 × MON 88017 × 59122 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins are similar in the five-event stack maize and in the single events except for CP4 EPSPS and PAT which showed the expected higher levels in the stack resulting from the combination of MON 87427 and MON 88017 (producing CP4 EPSPS) and 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 foreseen interactions at the biological level are between the Cry proteins in susceptible insects (Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1 and Cry35Ab1), 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[14]

Application EFSA-GMO-BE-2013-118 presents data on agronomic and phenotypic characteristics, as well as on forage and grain composition of the five-event stack maize MON 87427 × MON 89034 ×1507 × MON 88017 × 59122 derived from field trials performed in the US in 2010 (Table 6).

[14] Dossier: Part II – Sections A3.1 and A3.2; additional information: 2/10/2015, 13/6/2016, 11/7/2016 and 1/9/2016.

Table 6: Overview of comparative analysis studies with maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 provided in application EFSA-GMO-BE-2013-118

Study focus	Study details	Comparator	Commercial non-GM maize reference varieties[(a)]
Agronomic and phenotypic characteristics	Field trials, 2010, US, nine locations	Maize EXP262	Twenty-eight
Compositional analysis	Field trials, 2010, US, eight locations	Maize EXP262	Twenty-four

non-GM: non-genetically modified.
(a): Four different varieties were grown at each location.

The field trials were conducted in major maize growing areas of the US,[15] 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: maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122, a non-GM comparator (maize EXP262) and four non-GM maize reference varieties, all treated (sprayed) with plant protection products according to local requirements, and maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 treated with the intended herbicides, in addition to plant protection products. A total of 28[16] and 24[17] non-GM maize reference varieties were included in the agronomic and phenotypic and compositional analysis, respectively. The comparator used in the agronomic/phenotypic and compositional field trials is a non-GM maize line (EXP262 [= HCL301 × LH287]) with a genetic background similar to that of the five-event stack maize, as documented by the pedigree, and was therefore considered to be an appropriate non-GM comparator by the GMO Panel.

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, 2010d, EFSA GMO Panel, 2011a). This included, for each of the two treatments of maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122, 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 maize reference varieties). The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).[18]

3.3.2.2. Agronomic and phenotypic characteristics

Agronomic and phenotypic characteristics tested under field conditions[19]

The agronomic and phenotypic endpoints evaluated in the 2010 field trials were: early stand count, days to 50% pollen shed, days to 50% silking, stay green, ear height, plant height, dropped ear count, stalk lodged plants, root lodged plants, final stand count, grain moisture, test weight, yield, and visually observable responses to naturally occurring diseases, arthropod damage and abiotic stressors.

Two of the 16 endpoints evaluated (dropped ear count and root lodged plants) were not subject to a formal statistical analysis due to the lack of variability in the data.

[15] The sites for the agronomic and phenotypic field trials were in Jackson (AR); Hardin (IA); Greene (IA); Clinton (IL); Stark (IL); Boone (IN); Pawnee (KS); York (NE); and Miami (OH). The sites for the compositional analysis field trials were in Jackson (AR); Greene (IA); Jefferson (IA); Clinton (IL); Stark (IL); Boone (IN); Pawnee (KS); and York (NE).

[16] Burrus 645, Cornbelt ×6043, DKC 60-15, DKC 61-50, DKC62-30, Fielder's Choice NG6778, Fontanelle 4924, H-9180, iCorn 110.M7, 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 N64Z, NK N72-G8, Pioneer 32B81, Pioneer 32T16, Pioneer 33T56, Specialty 4672A, Stewart S518, Stewart S588 and Triumph 1416.

[17] Burrus 645, Cornbelt x6043, DKC60-15, DKC61-50, Fielder's Choice NG6778, Fontanelle 4924, H-9180, iCorn 110.M7, Legacy L6600, Legacy L6673, Lewis 7007, Midland Phillips 799, Midland Phillips 7B15P, Midwest Genetics 78130, Mycogen 2M746, NC+ 5411, NK N72-G8, Pioneer 32B81,Pioneer 32T16, Pioneer 33T56, Specialty 4672A, Stewart S518, Stewart S588 and Triumph 1416.

[18] 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).

[19] Dossier: Part II – Section A3.4; additional information: 2/10/2015, 13/6/2016, 11/7/2016 and 1/9/2016.

Statistically significant differences between the five-event stack maize not treated with the intended herbicides and the non-GM comparator were identified for: early stand count, ear height, plant height, stalk lodged plants, grain moisture and test weight; and between the five-event stack maize treated with the intended herbicides and the non-GM comparator for: days to 50% pollen shed, days to 50% silking, ear height, plant height, stalk lodged plants, grain moisture and test weight. Except for stalk lodged plants, for which the test of equivalence could not be applied (because the variation between the non-GM reference varieties was estimated to be zero), all these endpoints fell under equivalence category I.

Given the magnitude of the observed differences, the outcome of the equivalence test and the nature of the endpoints, the GMO Panel considered that none of the agronomic and phenotypic differences between the five-event stack maize and the non-GM comparator were relevant for further assessment.

3.3.2.3. Compositional analysis[20]

Forage and grain harvested from the field trials in the US in 2010 (Table 6) were analysed for 78 different constituents (9 in forage and 69 in grain), including the key constituents recommended by the OECD (OECD, 2002). For 15 grain components,[21] more than 50% of the observations were below the limit of quantification. The statistical analysis was applied to the remaining 63 constituents (9 in forage[22] and 54 in grain[23]).

The test of equivalence could not be applied to acid detergent fibre (ADF) in forage because the variation between the non-GM commercial reference varieties was estimated to be zero. A significant difference for ADF in forage was identified between MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 (treated) and the non-GM comparator.[24]

The combination of test of difference and test of equivalence could be applied to the remaining 62 endpoints (forage and grain), with the following results:

- Statistically significant differences between maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 (not treated) and the non-GM comparator were identified for 47 endpoints.[25] All the endpoints fell under equivalence category I or II.
- Statistically significant differences between maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 (treated) and the non-GM comparator were identified for 50 endpoints.[26] All the endpoints fell under equivalence category I except for thiamin levels which fell under category III.[27]

The GMO Panel assessed all the compositional differences between maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and the non-GM comparator. After considering the known biological role of the compounds, the outcome of the equivalence test and the magnitude of the

[20] Dossier: Part II – Section A3.3.

[21] Sodium, 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), heptadecanoic (C17:0), heptadecenoic (C17:1), γ-linolenic (C18:3), eicosadienoic (C20:2), eicosatrienoic (C20:3) and arachidonic (C20:4).

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

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

[24] Mean levels of ADF in forage (% DM): 24.38 (non-GM comparator); 25.80 (treated GM maize). DM: dry matter.

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

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

[27] Results for thiamin in grain (mg/kg DM) were as follows. Mean level for the non-GM comparator: 2.53; mean level for the treated GM maize: 2.34; equivalence limits: (2.36, 4.19). DM: dry matter.

changes observed, the GMO Panel did not identify any need for further food/feed safety assessment except for the change in thiamin levels.

3.3.2.4. Conclusions of the comparative analysis

The GMO Panel concludes that, except for thiamin, none of the differences identified between maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and the non-GM comparator in forage and grain composition and agronomic and phenotypic characteristics needs further assessment regarding food and feed safety. The nutritional impact of the reduced thiamin levels in maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 is further discussed in Section 3.3.3.5.

Moreover, none of the differences identified in the agronomic and phenotypic characteristics tested between maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and the non-GM comparator needs further assessment regarding its potential environmental impact.

3.3.3. Food and feed safety assessment

3.3.3.1. Effects of processing

Processed products

Maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 will undergo existing production processes used for conventional maize. No novel production process is envisaged.

Newly expressed proteins

Effects of heat treatment on newly expressed proteins Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS have been previously assessed by the GMO Panel in the context of the single maize events (see Table 2).

3.3.3.2. Toxicology

Toxicological assessment of newly expressed proteins[28]

Eight proteins (Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS) are newly expressed in maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 (Section 3.3.1).

The GMO Panel has previously assessed the safety of the Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS proteins individually in the context of the single maize events, and no safety concerns were identified for humans and animals (Table 2). The GMO Panel is not aware of any new information that would change this conclusion.

The potential for a functional interaction between the proteins newly expressed in maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 was assessed with regard to human and animal health. The two enzymes PAT and CP4 EPSPS catalyse distinct biochemical reactions and act on unrelated substrates in the plant with high substrate specificity. The six insecticidal proteins (Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1 and Cry35Ab1) 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 between the newly expressed proteins relevant for food and feed safety. Since the individual proteins are considered safe for humans and animals, the same conclusion can be extended to their presence in the five-event stack maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122.

The GMO Panel concludes that there are no safety concerns to human and animal health related to the newly expressed proteins Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, CP4 EPSPS and PAT in the five-event stack maize.

[28] Dossier: Part II – Section A4.2.

Toxicological assessment of components other than newly expressed proteins[29]

The five-event stack maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 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

No animal studies with food/feed derived from maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 were provided by the applicant (e.g. 90-day toxicity feeding studies in rodents or feeding studies in young rapidly growing animal species).

The decrease in thiamin was considered not to give rise to a substantial modification in the composition of the food and feed derived from the five-event stack maize (Sections 3.3.2 and 3.3.3.5). Furthermore, no indication for potential occurrence of unintended effects based on the molecular, compositional or phenotypic analyses, and no indication of possible interactions between the events were identified (Sections 3.3.1 and 3.3.2). Therefore, no animal studies on the food and feed derived from maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 are 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 of 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[30]

For allergenicity, the GMO Panel has previously evaluated the safety of the Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS proteins individually, and no concerns on allergenicity were identified in the context of the applications assessed (see 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 the five-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 Panel has previously evaluated the safety of the Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins and no concerns on adjuvanticity were identified in the context of the applications assessed (see Table 2). The levels of individual Bt proteins in the five-event stack maize are similar to those in the respective single maize events (see Appendix A). 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 the five-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[31]

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[32] (OECD, 2002). Therefore, the GMO Panel did not request experimental data to analyse the allergen repertoire of GM maize.

[29] Dossier: Part II – Section A4.3.
[30] Dossier: Part II – Sections A5.1 and A5.3.
[31] Dossier: Part II – Section A5.2.
[32] 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.

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 (see 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 five-event stack maize with respect to that derived from the non-GM comparator.

3.3.3.5. Nutritional assessment of GM food/feed

The intended traits of maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 are herbicide tolerance and insect resistance, with no intention to alter nutritional parameters. Comparison of the composition of the five-event stack maize with that of the non-GM comparator and the non-GM commercial reference varieties identified a decrease of thiamin (approximately 8%) in grains of the five-event stack maize that required further nutritional assessment (see Section 3.3.2).

The nutritional implications in humans of the decrease of thiamin were assessed based on the fact that grain and grain-based products are among the main contributors to thiamin intake across different age classes. As an example, the contribution of this food group can represent up to 40% of the total intake of thiamin in the adult population (EFSA NDA Panel, 2016). The GMO Panel performed a detailed evaluation of the particular contribution of maize and maize-based products to the total intake of thiamin using consumption data from the EFSA Comprehensive Food Consumption database[33] (EFSA, 2011a) and the EFSA nutrient composition database.[34] This assessment showed that the average contribution of maize and maize-based products to the thiamin intake is rather limited, with an estimated range in adults between 0.2% and 5.5% across different European countries.

Considering the extent of the decrease and, above all, the limited role of maize and maize-based products in the intake of thiamin, the GMO Panel concludes that the nutritional impact of the foods derived from the five-event stack maize is similar to that expected from the non-GM comparator and non-GM commercial reference varieties.

Regarding feed, thiamin is synthesized in the gastrointestinal tract of some animals and, moreover, the feed is balanced for vitamins with vitamin premixes. Therefore, the GMO Panel also considers that the nutritional impact of the feed derived from the five-event stack maize is similar to that expected from the non-GM comparator and non-GM commercial varieties.

3.3.3.6. Conclusion of the food and feed safety assessment

The newly expressed proteins Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS in maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 do not raise safety concerns for human and animal health. No interactions between these newly expressed 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 × 1507 × MON 88017 × 59122, or regarding the overall allergenicity of the five-event stack maize. The five-event stack maize is as safe and nutritious as the non-GM comparator and the non-GM commercial reference varieties tested.

3.3.4. Environmental risk assessment

Considering the scope of application EFSA-GMO-BE-2013-118 (which excludes cultivation), the ERA of the maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 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 GM maize grains during transportation and/or processing (EFSA GMO Panel, 2010a).

3.3.4.1. Persistence and invasiveness of the GM plant[35]

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

[33] Thirteen dietary surveys from nine countries were used in the assessment; the dietary surveys were classified according to FoodEx2 classification (EFSA, 2011b).
[34] EFSA nutrient composition database was compiled as a deliverable of the EFSA procurement project 'Updated food composition database for nutrient intake' (Roe et al., 2013).
[35] Dossier: Part II – Section E3.1.

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 grains 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).

As described in Section 3.3.2.2, field trials were performed in the US in the 2010 growing season to assess the agronomic/phenotypic characteristics of the five-event stack maize in comparison with the non-GM comparator. The data showed no changes in agronomic and phenotypic plant characteristics that would indicate altered fitness, persistence and invasiveness of the five-event stack maize.

Considering the agronomic and phenotypic characteristics of the five-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 five events of which it is composed. Should these plants be exposed to glyphosate- or glufosinate-containing herbicides, or infested by insect pests that are susceptible to the Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1 or Cry35Ab1 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 five-event stack maize or maize with comparable properties. Therefore, the GMO Panel concludes that it is unlikely that maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 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.

3.3.4.2. Potential for gene transfer[36]

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 (see 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 independent updated bioinformatic analysis for each of the single events in order to assess possibility for HGT by HR (Section 3.2).

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 analysis 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 event 1507 revealed that due to codon optimisation of the *cry1F* and *pat* gene there was no sequence identity with bacterial genes and thus no indication for HR.

Bioinformatic analysis of event MON 88017 revealed that the two genetic elements encoding for Cry3Bb1 and PAT were codon-optimised and did not provide sufficient sequence identity to bacterial DNA. However, four qualified alignments were detected with T-DNA from Ti plasmids of *A. tumefaciens*. No paired alignments and thus no potential to facilitate double HR were identified. Gene replacements of T-DNA-sequences on natural Ti plasmids, which are extremely unlikely due to an

[36] Dossier: Part II – Sections E3.1 and E.3.2.

expected absence of very low abundance of *A. tumefaciens* in the main receiving environments, i.e. the gastrointestinal tract, would not confer any new trait or selective advantage to bacterial recipients.

Bioinformatic analysis of event 59122 revealed no sequence identity with bacterial DNA which would facilitate HR, because the original bacterial genes encoding for Cry34Ab1, Cry35Ab1 and the PAT protein were all codon-optimised for expression in plants.

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 five-event stack maize. This is consistent with its previous assessments of maize events MON 87427, MON 89034, 1507, MON 88017 and 59122.

Plant-to-plant gene transfer

Considering the scope of application EFSA-GMO-BE-2013-118 and the biology of maize, the potential of occasional feral GM maize plants originating from grain import spills to transfer recombinant DNA to sexually cross-compatible plants and the environmental consequences thereof were considered. 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 plants 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 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[37]

Interactions might occur between different Cry proteins. Whether such an interaction takes place depends on the arthropod species tested (EcoΣtat, 2014; De Schrijver et al., 2015). Considering the scope of application EFSA-GMO-BE-2013-118, and the low level of exposure of the environment to this GM maize, potential interactions of occasional feral GM plants arising from spilled grains with 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[38]

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, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1 and/or Cry35Ab1 proteins is likely to be very low.

The GMO Panel also evaluated whether the expressed Cry 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 the manure and faeces will take place because of microbiological proteolytic activity. In addition, there will be further degradation of the expressed Cry proteins in soil,

[37] Dossier: Part II – Section E3.3.
[38] Dossier: Part II – Section E3.4.

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 microorganisms.

Considering the scope of the application, it can be concluded that the exposure of potentially sensitive non-target organisms to the Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1 and/or Cry35Ab1 proteins is likely to be very low and that the risks related to interactions with non-target organisms are therefore of no relevance.

3.3.4.5. Interactions with abiotic environment and biochemical cycles[39]

Considering the scope of application EFSA-GMO-BE-2013-118, and the low level of exposure to the environment, potential interactions of spilled grains or occasional feral maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 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 maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 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, Cry2Ab2, Cry1F, Cry3Bb1 Cry34Ab1 and/or Cry35Ab1 proteins, or exposed to glyphosate- and/or glufosinate-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-2013-118, interactions with the biotic and abiotic environment are not considered to be relevant issues. Risks associated with an unlikely but theoretically possible HGT of recombinant DNA from the five-event stack maize 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 exposure levels, the GMO Panel concludes that maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 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 five-event stack maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122

No new data on the single maize events MON 87427, MON 89034, 1507, MON 88017 and 59122 leading to a modification of the original conclusions on their safety were identified.

The combination of maize events MON 87427, MON 89034, 1507, MON 88017 and 59122 in the five-event stack maize did not give rise to issues pertaining to the molecular, agronomic/phenotypic or compositional characteristics of the five-event stack maize that would be of concern for food and feed safety and nutrition.

The newly expressed proteins in the five-event 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 × 1507 × MON 88017 × 59122. Comparison of the levels of the newly expressed proteins between the five-event stack maize and those of the single maize events did not reveal an interaction at the protein expression level.

Considering the combined events and the outcome of the comparative analysis, the routes of exposure and limited exposure levels, the EFSA GMO Panel concludes that maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 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 the five-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 maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 is as

[39] Dossier: Part II – Section E3.6.

safe and as nutritious as the non-GM comparator and the non-GM commercial reference varieties 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).

For those subcombinations for which no specific data have been submitted and which have not been previously assessed by the GMO Panel (see Table 7), the risk assessment takes as its starting point the assessment of the single maize events MON 87427, MON 89034, 1507, MON 88017 and 59122 and uses the data generated for the five-event stack maize, as well as all the additional data available on maize subcombinations of these single events that were previously assessed by the GMO Panel (see Table 2).

3.4.1. Subcombinations previously assessed

The GMO Panel has previously assessed the two-event maize stacks 1507 × 59122 and MON 89034 × MON 88017 and no safety concerns were identified (EFSA GMO Panel, 2009b, EFSA GMO Panel, 2010b). In addition, the GMO Panel has already assessed the four-event stack maize MON 89034 × 1507 × MON 88017 × 59122 and all its subcombinations independently of their origin (see Table 2) and did not identify any safety concerns (EFSA GMO Panel, 2010c). Moreover, no scientific information relevant to the risk assessment of these maize stacks became available since the validation of application EFSA-GMO-BE-2013-118. Consequently, the GMO Panel considers that its previous conclusions on these subcombinations remain valid.

3.4.2. Subcombinations not previously assessed

Fourteen out of 25 subcombinations included in the scope of this application have not been previously assessed by the GMO Panel, and no experimental data were provided for these maize stacks (see Table 7).

Table 7: Maize stacks not previously assessed and covered by the scope of application EFSA-GMO-BE-2013-118

Degree of stacking	Events
Four-event stack maize	MON 87427 × 1507 × MON 88017 × 59122
	MON 87427 × MON 89034 × 1507 × 59122
	MON 87427 × MON 89034 × 1507 × MON 88017
	MON 87427 × MON 89034 × MON 88017 × 59122
Three-event stack maize	MON 87427 × 1507 × 59122
	MON 87427 × 1507 × MON 88017
	MON 87427 × MON 88017 × 59122
	MON 87427 × MON 89034 × 1507
	MON 87427 × MON 89034 × 59122
	MON 87427 × MON 89034 × MON 88017
Two-event stack maize	MON 87427 × 1507
	MON 87427 × 59122
	MON 87427 × MON 88017
	MON 87427 × MON 89034

3.4.2.1. Stability of the events

The genetic stability of the inserted DNA over multiple generations in the five single maize events was demonstrated previously (see Table 2). Integrity of the events was demonstrated in the five-event stack maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 (Section 3.3.1.2) and the previously assessed maize subcombinations (EFSA GMO Panel, 2009b, EFSA GMO Panel, 2010b,c). The GMO Panel finds no reasons to expect the loss of integrity of the events in the maize subcombinations not previously assessed (see Table 7).

3.4.2.2. Expression of the events

The GMO Panel assessed whether the combination of any of the five 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 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 five-event stack maize. The levels were similar in the five-event stack maize and in the single events except for CP4 EPSPS and PAT, which showed the expected higher levels in the stack resulting from the combination of the MON 87427 and MON 88017 events both producing CP4 EPSPS protein, and 1507 and 59122 events 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 maize stacks 1507 × 59122 and MON 89034 × MON 88017 (EFSA GMO Panel, 2009b, EFSA GMO Panel, 2010b) and the four-event stack maize MON 89034 × 1507 × MON 88017 × 59122 (EFSA GMO Panel, 2010c) were similar to those observed in each of the single maize events or showed the expected higher levels for PAT resulting from the combination of 1507 and 59122 events both producing PAT protein in the four-event stack maize MON 89034 × 1507 × MON 88017 × 59122. This confirms that interactions affecting expression levels of the newly expressed proteins are not expected in the 14 maize subcombinations not previously assessed and included in the scope of application EFSA-GMO-BE-2013-118.

3.4.2.3. Potential functional interactions between the events

The GMO Panel assessed the potential interactions between events, due to their combination in the subcombinations not previously assessed and included in the scope of application EFSA-GMO-BE-2013-118 (Table 7), 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 the 14 subcombinations not previously assessed relevant for the food and feed and environmental safety. The GMO Panel took into account all the intended and potential unintended effects considered in the assessment of the five single events, the previously assessed subcombinations (Table 2) and the five-event stack maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122. It was concluded that none of these effects would raise safety concerns when combined in any of these maize subcombinations.[40] Therefore, the GMO Panel is of the opinion that no additional data are needed to complete the assessment of subcombinations from the five-event stack maize.

3.4.3. Conclusion

Since no new safety concerns were identified for the previously assessed two-event maize stacks 1507 × 59122 and MON 89034 × MON 88017 as well as the four-event stack maize MON 89034 × 1507 × MON 88017 × 59122 and all its subcombinations, the GMO Panel considers that its previous conclusions on these maize subcombinations remain valid. For the remaining 14 subcombinations included in the scope of application EFSA-GMO-BE-2013-118 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 subcombinations are therefore expected to be as safe as the single maize events, the previously assessed two-event maize stacks 1507 × 59122 and MON 89034 × MON 88017 and the four-event stack maize MON 89034 × 1507 × MON 88017 × 59122 including all its subcombinations as well as the five-event stack maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122.

[40] 15th GMO Panel meeting (Annex 1 of the minutes: http://www.efsa.europa.eu/sites/default/files/event/170517-m.pdf)

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 five-event stack maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 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 × 1507 × MON 88017 × 59122 into the market.

The two-event maize stacks 1507 × 59122 and MON 89034 × MON 88017 as well as the four-event stack maize MON 89034 × 1507 × MON 88017 × 59122 and all its subcombinations have been previously assessed and no safety concerns were identified. The 14 subcombinations not previously assessed and included in the scope of application EFSA-GMO-BE-2013-118 are expected to be as safe as the single maize events, the previously assessed maize subcombinations and the five-event stack maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122. Therefore, the GMO Panel considers that post-market monitoring of maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and its subcombinations is not necessary.

3.5.2. Post-market environmental monitoring[41]

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 content of the PMEM plan provided by the applicant (EFSA GMO Panel, 2011b).

As the ERA did not identify potential adverse environmental effects from the five-event stack maize, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for the five-event stack maize includes: (1) the description of a monitoring approach involving operators (federations involved in import and processing), reporting to the applicant, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment; (2) a coordinating system established by EuropaBio for the collection of information recorded by the various operators; and (3) the 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 GMO Panel considers that the scope of the PMEM plan provided by the applicant is consistent with the scope of maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122. As the scope does not cover cultivation and potential adverse environmental effects from the five-event stack maize were not identified, 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 five-event stack maize does not include any provisions for the 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 five single maize events MON 87427, MON 89034, 1507, MON 88017 and 59122 that would lead to a modification of the original conclusions on their safety were identified.

The combination of the events MON 87427, MON 89034, 1507, MON 88017 and 59122 in the five-event stack maize did not give rise to issues relating to molecular, agronomic/phenotypic and compositional characteristics regarding food and feed safety or nutrition. The newly expressed proteins in the five-event stack maize did not raise concerns for human and animal health. Maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 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 ×

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

1507 × MON 88017 × 59122 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 × 1507 × MON 88017 × 59122 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 maize stacks 1507 × 59122 and MON 89034 × MON 88017 and the four-event stack maize MON 89034 × 1507 × MON 88017 × 59122 and its subcombinations 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. For the additional 14 maize subcombinations included in the scope of application EFSA-GMO-BE-2013-118 for which no experimental data have been provided (Table 7), the GMO Panel assessed possible interactions between the events, and concludes that combinations of the events MON 87427, MON 89034, 1507, MON 88017 and 59122 would not raise safety concerns in these maize subcombinations. These subcombinations are therefore expected to be as safe and as nutritious as the single maize events, all the previously assessed subcombinations and the five-event stack maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122.

Given the absence of safety concerns for food and feed derived from the five-event stack maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and all its subcombinations, 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 five-event stack maize and the already assessed subcombinations. 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 five-event stack maize does not include any provisions for the 14 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 26 November 2013 concerning a request for placing on the market of genetically modified maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 submitted by Monsanto Europe S.A./N.V. in accordance with Regulation (EC) No 1829/2003 (application reference EFSA-GMO-BE-2013-118).
2) Acknowledgement letter dated 3 December 2013 from EFSA to the Competent Authority of Belgium.
3) Letter from EFSA to applicant dated 17 January 2014 requesting additional information under completeness check.
4) Letter from applicant to EFSA received on 17 February 2014 providing additional information under completeness check.
5) Letter from EFSA to applicant dated 10 March 2014 delivering the 'Statement of Validity' of application EFSA-GMO-BE-2013-118 for placing on the market of genetically modified maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 submitted by Monsanto Europe S.A./N.V. in accordance with Regulation (EC) No 1829/2003.
6) Letter from EFSA to applicant dated 11 June 2015 requesting additional information and stopping the clock.
7) Letter from applicant to EFSA received on 6 July 2015 providing additional information.
8) Letter from EFSA to applicant dated 30 July 2015 requesting additional information and maintaining the clock stopped.
9) Letter from applicant to EFSA received on 2 October 2015 providing additional information.
10) Letter from EFSA to applicant dated 26 November 2015 requesting additional information and maintaining the clock stopped.
11) Letter from EFSA to applicant dated 11 December 2015 requesting additional information and maintaining the clock stopped.
12) Letter from applicant to EFSA received on 18 December 2015 providing additional information.
13) Letter from EFSA to applicant dated 8 March 2016 requesting additional information and maintaining the clock stopped.

14) Letter from applicant to EFSA, received on 8 June 2016 extending the timeline for submission of responses.
15) Letter from applicant to EFSA received on 13 June 2016 providing additional information.
16) Letter from EFSA to applicant dated 28 June 2016 requesting additional information and maintaining the clock stopped.
17) Letter from applicant to EFSA received on 11 July 2016 providing additional information.
18) Letter from applicant to EFSA received on 29 July 2016 providing additional information.
19) Email from EFSA to applicant, dated 29 July 2016, re-starting the clock.
20) Letter from EFSA to applicant dated 4 August 2016 requesting additional information and stopping the clock.
21) Letter from EFSA to applicant dated 11 August 2016 requesting additional information and maintaining the clock stopped.
22) Letter from applicant to EFSA received on 1 September 2016 providing additional information.
23) Letter from applicant to EFSA received on 15 September 2016 providing additional information.
24) Letter from EFSA to applicant dated 25 October 2016 requesting additional information and maintaining the clock stopped.
25) Letter from applicant to EFSA received on 24 November 2016 providing additional information.
26) Letter from applicant to EFSA received on 25 November 2016 providing additional information. This package included spontaneous information.
27) Email from EFSA to applicant, dated 29 November 2016, re-starting the clock from 25 November 2016.
28) Letter from applicant to EFSA received on 13 December 2016 providing Updated PMEM, Cartagena, Labelling and Summary of application spontaneously.
29) Letter from EFSA to applicant dated 11 April 2017 requesting additional information and stopping the clock.
30) Letter from EFSA to applicant dated 16 May 2017 requesting additional information and maintaining the clock stopped.
31) Letter from applicant to EFSA received on 6 June 2017 providing additional information.
32) Letter from applicant to EFSA received on 20 June 2017 providing additional information.
33) Email from EFSA to applicant, dated 20 June 2016, re-starting the clock.

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Abbreviations

ADF	acid detergent fibre
CaMV	cauliflower mosaic virus
CTP	chloroplast transit peptide
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
IgE	immunoglobulin E
HGT	horizontal gene transfer
HR	homologous recombination
NDF	neutral detergent fibre
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PAT	phosphinothricin acetyltransferase
PMEM	post-market environmental monitoring
TDF	total detergent fibre
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 × 1507 × MON 88017 × 59122 (treated with glyphosate and glufosinate), MON 87427 (treated with glyphosate), MON 89034 (not treated), 1507 (treated with glufosinate), MON 88017 (treated with glyphosate) and 59122 (treated with glufosinate) from field trials performed in the US in 2010.

	MON 87427 × MON 89034 × 1507 × MON 88017 × 59122	MON 87427	MON 89034	1507	MON 88017	59122
Cry1A.105						
Leaf (V2–V4)	300[(a)] ± 61[(b)] (230–420)[(c)]		290 ± 82 (150–390)			
Root (V2–V4)	83 ± 27 (49–140)		56 ± 11 (42–76)			
Root (R5)	14 ± 3.3 (7.8–19)		12 ± 3.7 (7.5–20)			
Whole Plant (V10–V12)	58 ± 12 (38–81)		53 ± 9.1 (37–73)			
Forage (R5)	32 ± 8.6 (16–45)		23 ± 6.2 (9.2–32)			
Pollen (pollination)	14 ± 2.5 (10–19)		5.5 ± 4.3 (2.8–22)			
Grain (R6)	8.1 ± 2.4 (6.0–14)		5.9 ± 1.0 (4.3–7.9)			
Cry2Ab2						
Leaf (V2–V4)	260 ± 78 (110–390)		220 ± 52 (92–350)			
Root (V2–V4)	40 ± 13 (23–72)		32 ± 8.7 (22–57)			
Root (R5)	17 ± 7.9 (3.1–30)		19 ± 7.6 (7.7–33)			
Whole Plant (V10–V12)	50 ± 11 (29–66)		32 ± 5.7 (18–39)			
Forage (R5)	39 ± 10 (17–58)		32 ± 8.5 (16–53)			
Pollen (pollination)	0.33 ± 0.11 (0.19–0.66)		0.31 ± 0.12 (0.18–0.52)			
Grain (R6)	1.8 ± 0.40) (1.2–2.8)		1.6 ± 0.61 (0.96–3.8)			
Cry1F						
Leaf (V2–V4)	20 ± 3.7 (15–28)			17 ± 7.1 (11–42)		
Root (V2–V4)	10 ± 3.3 (5.0–17)			8.8 ± 2.9 (4.6–15)		
Root (R5)	2.7 ± 0.69 (1.5–3.8)			2.8 ± 0.67 (1.7–3.8)		
Whole Plant (V10–V12)	9.6 ± 1.6 (6.9–13)			8.3 ± 2.2 (5.3–12)		
Forage (R5)	5.0 ± 1.2 (3.1–7.3)			4.8 ± 1.1 (3.2–6.4)		
Pollen (pollination)	12 ± 1.5 (9.6–16)			11 ± 1.4 (8.8–14)		
Grain (R6)	2.0 ± 0.33 (1.6–2.8)			1.8 ± 0.22 (1.4–2.1)		

	MON 87427 × MON 89034 × 1507 × MON 88017 × 59122	MON 87427	MON 89034	1507	MON 88017	59122
Cry3Bb1						
Leaf (V2–V4)	260 ± 39 (200–320)				250 ± 40 (190–320)	
Root (V2–V4)	150 ± 32 (97–220)				150 ± 35 (110–210)	
Root (R5)	51 ± 14 (31–84)				77 ± 17 (44–110)	
Whole Plant (V10–V12)	120 ± 30 (76–200)				110 ± 20 (69–140)	
Forage (R5)	50 ± 11 (30–69)				50 ± 14 (33–75)	
Pollen (pollination)	14 ± 2.3 (11–19)				12 ± 2.1 (4.4–14)	
Grain (R6)	7.8 ± 1.1 (6.1–9.6)				9.5 ± 0.87 (7.5–11)	
Cry34Ab1						
Leaf (V2–V4)	53 ± 16 (27–85)					40 ± 9.1 (24–54)
Root (V2–V4)	67 ± 26 (26–130)					62 ± 28 (21–120)
Root (R5)	39 ± 15 (12–76)					38 ± 13 (11–58)
Whole Plant (V10–V12)	61 ± 17 (29–96)					84 ± 52 (33–210)
Forage (R5)	99 ± 23 (66–160)					73 ± 23 (47–140)
Pollen (pollination)	72 ± 7.2 (57–88)					92 ± 13 (74–120)
Grain (R6)	35 ± 4.3 (29–42)					29 ± 5.3 (21–39)
Cry35Ab1						
Leaf (V2–V4)	19 ± 6.2 (11–33)					16 ± 5.3 (5.3–27)
Root (V2–V4)	18 ± 8.0 (6.2–36)					19 ± 8.1 (3.9–34)
Root (R5)	3.0 ± 1.6 (0.69–7.4)					3.4 ± 1.8 (0.63–6.5)
Whole Plant (V10–V12)	18 ± 4.1 (7.8–26)					15 ± 3.9 (8.6–24)
Forage (R5)	12 ± 3.2 (6.9–18)					11 ± 3.3 (6.7–20)
Pollen (pollination)	< LOQ ± NA (< LOD–0.65)					<LOQ ± NA (<LOD–0.67)
Grain (R6)	0.55 ± 0.11 (0.39–0.80)					0.62 ± 0.17 (0.42–0.86)

	MON 87427 × MON 89034 × 1507 × MON 88017 × 59122	MON 87427	MON 89034	1507	MON 88017	59122
PAT						
Leaf (V2–V4)	8.4 ± 2.5 (4.0–14)			1.9 ± 0.53 (1.1–2.8)		8.0 ± 1.7 (4.9–11)
Root (V2–V4)	1.7 ± 0.62 (0.66–2.9)			0.43 ± 0.31 (0.083–1.5)		1.2 ± 0.37 (0.51–1.7)
Root (R5)	0.42 ± 0.19) (0.10–0.72)			0.15 ± 0.053 (0.063–0.25)		0.26 ± 0.14 (0.061–0.53)
Whole Plant (V10–V12)	4.3 ± 1.4 (2.8–8.0)			1.1 ± 0.45 (0.57–2.2)		3.2 ± 1.1 (1.7–6.0)
Forage (R5)	(0.85 ± 0.30) (0.17–1.1)			0.26 ± 0.088 (0.15–0.41)		0.76 ± 0.31 (0.19–1.2)
Pollen (pollination)	< LOD[d]			< LOD		< LOD
Grain (R6)	< LOD			< LOD		< LOD
CP4 EPSPS						
Leaf (V2–V4)	770 ± 100 (550–910)	780 ± 170 (540–1100)			210 ± 33 (150–270)	
Root (V2–V4)	200 ± 53 (130–320)	130 ± 35 (85–230)			57 ± 12 (41–78)	
Root (R5)	77 ± 28 (26–160)	60 ± 21 (30–100)			14 ± 5.1 (6.9–26)	
Whole Plant (V10–V12)	380 ± 58 (270–480)	320 ± 56 (240–430)			80 ± 14 (49–100)	
Forage (R5)	190 ± 50 (100–270)	140 ± 57 (62–270)			36 ± 13 (23–67)	
Pollen (pollination)	310 ± 66 (220–440)	< LOQ[e]			180 ± 47 (62–290)	
Grain (R6)	7.6 ± 1.1 (5.8–11)	4.9 ± 1.2 (2.7–7.1)			4.2 ± 1.1 (2.5–6.6)	

(a): Mean.
(b): Standard deviation.
(c): Range.
(d): LOD: limit of detection; LOQ: limit of quantification.
(e): Due to specific insert design, little to no CP4 EPSPS protein is expected to be produced in pollen.