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Assessment of genetically modified maize MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9 and subcombinations independently of their origin for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2013-113)

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Abstract

Maize MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9 (five-event stack maize) was produced by conventional crossing to combine five single events: MON 89034, 1507, MON 88017, 59122 and DAS-40278-9. The GMO Panel previously assessed the 5 single maize events and 11 of their subcombinations and did not identify safety concerns. No new data on the single maize events or their 11 subcombinations that could modify the original conclusions on their safety were identified. The molecular characterisation, comparative analysis (agronomic, phenotypic and compositional characteristics) and the outcome of the toxicological, allergenicity and nutritional assessment indicates that the combination of the single maize events and of the newly expressed proteins in the five-event stack maize does not give rise to food and feed safety and nutritional concerns. The GMO Panel concludes that the five-event stack maize, as described in this application, is as safe as and nutritionally equivalent to its non-GM comparator and the non-GM reference varieties tested. In the case of accidental release of the five-event stack maize into the environment, this would not raise environmental safety concerns. The GMO Panel assessed the likelihood of interactions among the single events in the 14 maize subcombinations for which no experimental data were provided, and concludes that they are expected to be as safe as and nutritionally equivalent to the single events, the previously assessed subcombinations and the five-event stack maize. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of the five-event stack maize. No post-market monitoring of food/feed is considered necessary. The GMO Panel concludes that the five-event stack maize and its subcombinations are as safe as its non-GM comparator and the tested non-GM reference varieties with respect to potential effects on human and animal health and the environment.

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Summary

Following the submission of application EFSA-GMO-NL-2013-113 under Regulation (EC) No 1829/2003 from Dow Agrosciences, the Panel on Genetically Modified Organisms of the European Food Safety Authority (hereafter referred to as the 'GMO Panel') was asked to deliver a scientific opinion on genetically modified (GM) maize MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9 and its subcombinations independently of their origin (referred to hereafter as 'subcombinations'). The scope of application EFSA-GMO-NL-2013-113 is for the placing on the market of maize MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9 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 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9 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 the subcombinations that either have been or could be produced by conventional crossing through targeted breeding approaches, which can be bred, produced and marketed independently of the five-event stack maize, are risk assessed in Section 3.4 of the present scientific opinion.

In delivering its Scientific Opinion, the GMO Panel considered the data available on the single events, the five-event stack maize, 11 subcombinations (six-two-event stacks, four-three-event stacks and one-four-event stack), 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 89034 (expressing the Cry1A.105 and Cry2Ab2 proteins); 1507 (expressing the Cry1F and phosphinothricin acetyltransferase (PAT) proteins); MON 88017 (expressing the Cry3Bb1 and 5-enolpyruvylshikimate-3-phosphate synthase (CP4-EPSPS) proteins); 59122 (expressing the Cry34Ab1, Cry35Ab1 and PAT proteins); and DAS-40278-9 (expressing the aryloxyalkanoate dioxygenase 1 (AAD-1) protein). Herbicidal tolerance traits are achieved by the expression of AAD-1 protein from *Sphingobium herbicidovorans*, CP4 EPSPS protein from *Agrobacterium tumefaciens* sp. strain CP4, and PAT protein from *Streptomyces viridochromogenes*. Insecticidal resistance traits are achieved by the expression of Cry1A.105, Cry2Ab2 and Cry1F proteins from *Bacillus thuringiensis*, which confer protection against specific lepidopteran pests, and through the expression of Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins derived from *B. thuringiensis* that provides protection against corn rootworm (*Diabrotica* spp.) larval feeding.

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 GM plants and derived food and feed, the environmental risk assessment of GM plants and the post-market environmental monitoring (PMEM) of GM plants.

For application EFSA-GMO-NL-2013-113, previous assessments of the 5 single maize events MON 89034, 1507, MON 88017, 59122 and DAS-40278-9 and 11 subcombinations (six-two-event stacks, four-three-event stacks and one-four-event stack), provided a basis to evaluate the five-event stack maize and all its subcombinations. No concerns on their safety were identified by the GMO Panel in the previous assessments. 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 and feed were evaluated with respect to potential toxicity, allergenicity and nutritional characteristics. An evaluation of environmental impacts and the PMEM plan was also undertaken.

The molecular characterization data establish that the events stacked in maize MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9 have retained their integrity. Protein expression analyses show that the levels of the newly expressed proteins are similar in the five-event stack and in the single events or already assessed four-event stack. No indications of interactions that may affect the integrity of the events and the levels of the newly expressed proteins in this five-event stack maize are identified.

The comparative analysis of forage and grain composition and agronomic/phenotypic characteristics identifies no differences between maize MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9

and the non-GM comparator requiring further assessment for food and feed safety or environmental impact, except for the changes in levels of protein, glutamic acid, glycine, leucine, lysine, threonine, magnesium and manganese in grain and the endpoint disease incidence which were further assessed and not found to have a safety impact.

The molecular characterisation, comparative analysis (agronomic, phenotypic and compositional characteristics) and the outcome of the toxicological, allergenicity and nutritional assessment indicate that the combination of the single maize events and of the newly expressed proteins in the five-event stack maize does not give rise to food and feed safety and nutritional concerns. The GMO Panel concludes that maize MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9, as described in this application, is as safe as and nutritionally equivalent to its non-GM comparator and the non-GM reference varieties tested.

Considering the events combined and their potential interactions, the outcome of the comparative analysis, and the routes and levels of exposure, the GMO Panel concludes that maize MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9 would not raise safety concerns in the case of accidental release of viable GM maize grains into the environment.

Since no new safety concerns have been identified for the previously assessed 11 subcombinations (six-two-event stacks, four-three-event stack and one-four-event stack), 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-NL-2013-113 for which no experimental data have been provided, the GMO Panel assessed the possibility of interactions between the events and concludes that these combinations would not raise safety concerns. These subcombinations are therefore expected to be as safe as and nutritionally equivalent to the maize single events, the previously assessed subcombinations and the five-event stack maize.

Given the absence of safety concerns for foods and feeds from 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 PMEM plan and reporting intervals are in line with the intended uses of the five-event stack maize and its subcombinations.

Table of Contents

Abstract ... 1
Summary ... 3
1. Introduction ... 6
1.1. Background ... 6
1.2. Terms of Reference as provided by the requestor ... 6
2. Data and methodologies ... 6
2.1. Data ... 6
2.2. Methodologies ... 7
3. Assessment ... 7
3.1. Introduction ... 7
3.2. Updated information on the single events ... 9
3.3. Risk assessment of the five-event maize MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9 ... 9
3.3.1. Molecular characterisation ... 9
3.3.1.1. Genetic elements and their biological function ... 9
3.3.1.2. Integrity of the events in the five-event stack maize ... 11
3.3.1.3. Information on the expression of the inserts ... 11
3.3.1.4. Conclusion on molecular characterisation ... 12
3.3.2. Comparative analysis ... 12
3.3.2.1. Choice of comparator and production of material for the comparative analysis ... 12
3.3.2.2. Agronomic and phenotypic analysis ... 13
3.3.2.3. Compositional analysis ... 13
3.3.2.4. Conclusion on comparative analysis ... 15
3.3.3. Food and feed safety assessment ... 15
3.3.3.1. Effects of processing ... 15
3.3.3.2. Influence of Temperature and pH on newly expressed proteins ... 15
3.3.3.3. Toxicology ... 15
3.3.3.4. Allergenicity ... 16
3.3.3.5. Nutritional assessment of GM food and feed ... 17
3.3.3.6. Conclusion on food and feed safety assessment ... 18
3.3.4. Environmental risk assessment ... 18
3.3.4.1. Persistence and invasiveness of the GM plant ... 18
3.3.4.2. Potential for gene transfer ... 19
3.3.4.3. Interactions of the GM plant with target organisms ... 19
3.3.4.4. Interactions of the GM plant with non-target organisms ... 20
3.3.4.5. Interactions with abiotic environment and biogeochemical cycles ... 20
3.3.4.6. Conclusion on environmental risk assessment ... 20
3.3.5. Conclusion on the five-event stack maize MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9 ... 20
3.4. Risk assessment of the subcombinations ... 20
3.4.1. Subcombinations previously assessed ... 21
3.4.2. Subcombinations not previously assessed ... 21
3.4.2.1. Stability of the events ... 21
3.4.2.2. Expression of the events ... 21
3.4.2.3. Potential functional interactions between the events ... 22
3.4.3. Conclusion ... 22
3.5. Post-market monitoring ... 22
3.5.1. Post-market monitoring of GM food/feed ... 22
3.5.2. Post-market environmental monitoring ... 22
3.5.3. Conclusion on post-market monitoring ... 23
4. Overall conclusions and recommendations ... 23
Documentation provided to EFSA ... 23
References ... 24
Abbreviations ... 27
Appendix A – Protein expression data ... 29

1. Introduction

The scope of application EFSA-GMO-NL-2013-113 is for food and feed uses, import and processing in the European Union (EU) of the genetically modified (GM) herbicide-tolerant insect-resistant maize MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9 and all its subcombinations independently of their origin.

1.1. Background

On 6 February 2013, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands application EFSA-GMO-NL-2013-113 for authorisation of maize MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9 (hereafter referred to as 'the five-event stack maize') (Unique Identifier MON-89Ø34-3 × DAS-Ø15Ø7-1 × MON-88Ø17-3 × DAS-59122-7 × DAS-4Ø278-9), submitted by Dow AgroSciences (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003[1].

Following receipt of application EFSA-GMO-NL-2013-113, EFSA informed EU Member States and the European Commission, and made the application available to them. Simultaneously, EFSA published the summary of the application.[2]

EFSA checked the application for compliance with the relevant requirements of Regulation (EC) No 1829/2003 and, when needed, asked the applicant to supplement the initial application. On 2 October 2014, EFSA declared the application valid.

From the validity date, EFSA and its scientific Panel on Genetically Modified Organisms (hereafter referred to as 'the GMO Panel') endeavoured to respect a time limit of six months to issue a scientific opinion on application EFSA-GMO-NL-2013-113. Such time limit was extended whenever EFSA and/or its GMO Panel requested supplementary information to the applicant. According to Regulation (EC) No 1829/2003, any supplementary information provided by the applicant during the risk assessment was made available to the EU Member States and European Commission (for further details, see the section 'Documentation', below).

In accordance with Regulation (EC) No 1829/2003, EFSA consulted the nominated risk assessment bodies of EU Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC[3]. The EU Member States had three months to make their opinion known on application EFSA-GMO-NL-2013-113 as of date of validity.

1.2. Terms of Reference as provided by the requestor

According to Articles 6 and 18 of Regulation (EC) No 1829/2003, EFSA and its GMO Panel were requested to carry out a scientific risk assessment of the five event stack maize and all its subcombinations independently of their origin, for food and feed uses, import and processing.

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).

In addition to the present scientific opinion, EFSA was also asked to report on the particulars listed under Articles 6(5) and 18(5) of Regulation (EC) No 1829/2003, but not to give an opinion on them, because they pertain to risk management.[4]

2. Data and methodologies

2.1. Data

The GMO Panel based its scientific assessment of the five-event stack maize on the valid application EFSA-GMO-NL-2013-113, additional information provided by the applicant during the risk assessment, relevant scientific comments submitted by EU Member States and relevant peer-reviewed scientific publications.

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.
[2] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2013-00989
[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] These particulars can be found in the technical report by EFSA on the application EFSA-GMO-NL-2013-113, made available in the EFSA Register of Questions.

2.2. Methodologies

The GMO Panel conducted its assessment in line with the principles described in Regulation (EU) No 1829/2003, its applicable guidelines (i.e. EFSA GMO Panel, 2010a, 2011a,b) and explanatory notes (i.e. EFSA, 2017a,b) for the risk assessment of GM plants.

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

3. Assessment

3.1. Introduction

Application EFSA-GMO-NL-2013-113 covers the five-event stack maize MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9 and all its subcombinations independently of their origin (Table 1).

The term 'subcombination' refers to the specific combinations 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 the five-event stack maize 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 89034, 1507, MON 88017, 59122 and DAS-40278-9 that have either been, or could be produced by conventional crossing through targeted breeding approaches (EFSA GMO Panel, 2011a). These are maize stacks that can be bred, produced and marketed independently of the five-event stack maize. These subcombinations are risk assessed in the Section 3.4 of this scientific opinion.

The five-event stack maize was produced by conventional crossing to combine five single maize events: 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 5-enolpyruvylshikimate-3-phosphate synthase (CP4-EPSPS) proteins); 59122 (expressing the Cry34Ab1, Cry35Ab1 and PAT proteins); and DAS-40278-9 (expressing the aryloxyalkanoate dioxygenase 1 (AAD-1) protein).

Herbicidal tolerance traits are achieved by the expression of AAD-1 protein from *Sphingobium herbicidovorans*, CP4 EPSPS protein from *Agrobacterium tumefaciens* sp. strain CP4 and PAT protein from *Streptomyces viridochromogenes*. Insecticidal resistance traits are achieved by the expression of Cry1A.105, Cry2Ab2 and Cry1F proteins from *Bacillus thuringiensis*, which confer protection against specific lepidopteran pests, and through the expression of Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins derived from *B. thuringiensis* that provides protection against corn rootworm (*Diabrotica* spp.) larval feeding.

Table 1: Stacked maize events covered by the scope of application EFSA-GMO-NL-2012-113

Degree of stacking	Events
Five-event stack maize	MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9
Four-event stack maize	MON 89034 × 1507 × MON 88017 × 59122
	MON 89034 × 1507 × MON 88017 × DAS-40278-9
	MON 89034 × 1507 × 59122 × DAS-40278-9
	MON 89034 × MON 88017 × 59122 × DAS-40278-9
	1507 × MON 88017 × 59122 × DAS-40278-9

Degree of stacking	Events
Three-event stack maize	MON 89034 × 1507 × MON 88017
	MON 89034 × 1507 × 59122
	MON 89034 × 1507 × DAS-40278-9
	MON 89034 × MON 88017 × 59122
	MON 89034 × MON 88017 × DAS-40278-9
	MON 89034 × 59122 × DAS-40278-9
	1507 × MON 88017 × 59122
	1507 × MON 88017 × DAS-40278-9
	1507 × 59122 × DAS-40278-9
	MON 88017 × 59122 × DAS-40278-9
Two-event stack maize	MON 89034 × 1507
	MON 89034 × MON 88017
	MON 89034 × 59122
	MON 89034 × DAS-40278-9
	1507 × MON 88017
	1507 × 59122
	1507 × DAS-40278-9
	MON 88017 × 59122
	MON 88017 × DAS-40278-9
	59122 × DAS-40278-9

All five single maize events MON 89034, 1507, MON 88017, 59122 and DAS-40278-9 and 11 subcombinations (six-two-event stacks, four-three-event stack and one-four-event stack) have been previously assessed (see Table 2). No concerns for human and animal health, or environmental safety were identified.

Table 2: Single maize events and subcombinations of maize MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9 previously assessed by the GMO Panel

Event	Application or mandate	EFSA Scientific Opinion
MON 89034	EFSA-GMO-NL-2007-37	EFSA (2008)
1507	C/NL/00/10	EFSA (2004)
	C/ES/01/01	EFSA (2005a)
	EFSA-GMO-NL-2004-02	EFSA (2005b)
	EFSA-GMO-RX-1507	EFSA (2009a)
	EFSA-M-2012-0231[(a)]	EFSA GMO Panel (2012)
	EFSA GMO-RX-001	EFSA GMO Panel (2017a)
MON 88017	EFSA-GMO-NL-2005-27	EFSA (2009b)
59122	EFSA-GMO-NL-2005-12	EFSA (2007)
	EFSA-GMO-NL-2005-23	EFSA (2013)
DAS-40278-9	EFSA GMO NL 2010-89	EFSA GMO Panel (2016a)
MON 89034 × MON 88017	EFSA-GMO-NL-2007-39	EFSA (2010)
MON 89034 × 1507	EFSA-GMO-NL-2009-65	EFSA GMO Panel (2011c)
1507 × 59122	EFSA-GMO-NL-2005-15	EFSA (2009c)
MON 89034 × 1507 × MON 88017 × 59122 (and its 10 subcombinations)	EFSA-GMO-CZ-2008-62 EFSA-GMO-BE-2013-118	EFSA GMO Panel (2010c, 2011d); EFSA GMO Panel (2017b)

(a): Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2012-00712

3.2. Updated information on the single events[5]

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

The applicant clarified that the 1507 maize sequence reported for the five-event stack maize contained one silent nucleotide change in the insert sequence compared to the corrected original 1507 maize sequence (EFSA GMO Panel, 2018a,b). Analysis of the new sequencing data and bioinformatic analyses performed on the new sequence does not identify any need for further safety assessment.

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

Updated bioinformatic analyses on the junction regions for maize events MON 89034, 1507, MON 88017, 59122 and DAS-40278-9, using the methodology specified in the 2011 GMO Panel Guidance Document (EFSA GMO Panel, 2011a) confirm that no known endogenous genes were disrupted by any of the inserts.

Updated bioinformatic analyses of the amino acid sequence of the newly expressed Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT, CP4-EPSPS and AAD-1 proteins reveal no significant similarities to toxins and allergens. Updated bioinformatic analyses of the newly created open reading frames (ORFs) within the insert or spanning the junctions between the insert and genomic DNA indicate that the expression of an ORF showing significant similarities to toxins or allergens is highly unlikely.

In order to assess the possibility for horizontal gene transfer (HGT) by homologous recombination, the applicant performed a sequence identity analysis for maize events MON 89034, 1507, MON 88017, 59122 and DAS-40278-9, with microbial DNA. The likelihood and potential consequences of plant-to-bacteria gene transfer are described in Section 3.3.4.2.

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

3.3. Risk assessment of the five-event maize MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9

3.3.1. Molecular characterisation[6]

Possible interactions that would affect the integrity of the events, newly expressed proteins levels or the biological function conferred by the individual inserts are considered below.

3.3.1.1. Genetic elements and their biological function

Maize events MON 89034, 1507, MON 88017, 59122 and DAS-40278-9 were combined by conventional crossing to produce the five-event stack maize. The structure of the inserts in the five-event stack 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 the five-event stack maize 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 six Cry proteins in susceptible insects.

[5] Dossier: Part II Scientific information, Section 2.2.2., and additional information 22/4/2015, 13/10/2016, 21/10/2016, 25/1/2017, 7/2/2017, 24/11/2017, 12/3/2018, 31/10/2018 and 20/11/2018.

[6] Dossier: Part II Scientific information, Section 2, and additional information 7/2/2017.

Table 3: Genetic elements in the expression cassettes of the events stacked in maize MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9

Event	Promoter	5′ UTR	Transit peptide	Coding region*	Terminator
MON 89034	35S (CaMV)	CAB (*Triticum* sp.)	–	*cry1A.105* (*Bacillus thuringiensis*)	*Hsp17* (*Triticum* sp.)
	35S (FMV)	–	CTP (*Zea mays*)	*cry2Ab2* (*B. thuringiensis*)	*nos* (*Agrobacterium tumefaciens*)
1507[(a)]	*ubi*ZM1 (*Z. mays*)	–	–	*cry1F* (*B. thuringiensis*)	ORF25PolyA (*A. tumefaciens*)
	35S (CaMV)	–	–	*pat* (*Streptomyces viridochromogenes*)	*35S* (CaMV)
MON 88017	act1 (*Oryza sativa*)	–	CTP2 (*Arabidopsis thaliana*)	CP4 epsps (*Agrobacterium* sp.)	nos (*A. tumefaciens*)
	35S (CaMV)	CAB (*Triticum* sp.)	–	*cry3Bb1* (*B. thuringiensis*)	hsp17 (*Triticum* sp)
59122	*ubiZM1* (*Z. mays*)	–	–	cry34Ab1 (*B. thuringiensis*)	pinII (*Solanum tuberosum*)
	Wheat peroxidase (*Triticum aestivum*)	–	–	*cry35Ab1* (*B. thuringiensis*)	pinII (*S. tuberosum*)
	35S (CaMV)	–	–	*pat* (*S. viridochromogenes*)	35S (CaMV)
DAS-40278-9	*ZmUbi1* (*Z. mays*)	–	–	*aad-1* (*Sphingobium herbicidovorans*)	*ZmPer5 3′ UTR* (*Z. mays*)

CaMV: Cauliflower Mosaic Virus; FMV: Figwort Mosaic Virus.
*: All gene sequences are codon-optimised for expression in plants.
–: When no element was specifically introduced to optimise expression.
(a): Maize 1507 also contains partial fragments of the cry1F and pat genes at a single locus in the nuclear genome.

Table 4: Characteristics and intended effects of the events stacked in maize MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9

Event	Protein	Donor organism and biological function	Intended effects in GM plant
MON 89034	Cry1A.105	Based on genes from *Bacillus thuringiensis* subsp. *kurstaki* and subsp. *aizawai*. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes (Schnepf et al., 1998; Ellis et al., 2002)	Event MON89034 expresses a modified version of the Cry1A-type protein. Cry1A.105 is a protein toxic to certain lepidopteran larvae feeding on maize
	Cry2Ab2	Based on a gene from *Bacillus thuringiensis* subsp. kurstaki. *B. thuringiensis* an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes (Schnepf et al., 1998; Ellis et al., 2002)	Event MON 89034 expresses the Cry2Ab2, a protein toxic to certain lepidopteran larvae feeding on maize
1507	Cry1F	Based on a gene from *Bacillus thuringiensis* subsp. *aizawai*. B. *thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes (Schnepf et al., 1998; Ellis et al., 2002)	Event 1507 expresses a truncated version of the Cry1F protein. Cry1F is a protein toxic to certain lepidopteran larvae feeding on maize
	PAT	Based on a gene from *Streptomyces viridochromogenes* Tü494. Phosphinothricin-acetyl-transferase (PAT) enzyme acetylates L-glufosinate-ammonium (Thompson et al., 1987; Wohlleben et al., 1988; Eckes et al., 1989)	Event 1507 expresses the PAT protein which confers tolerance to glufosinate-ammonium-based herbicides (Droge-Laser et al., 1994)

Event	Protein	Donor organism and biological function	Intended effects in GM plant
MON 88017	CP4 EPSPS	Based on a gene from *Agrobacterium* strain CP4. 5-Enolpyruvylshikimate-3-phosphate synthase (EPSPS) is an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Herrmann, 1995)	Event MON 88017 expresses the bacterial CP4 EPSPS protein which confers tolerance to glyphosate-based herbicides as it has lower affinity towards glyphosate than the plant endogenous enzyme
	Cry3Bb1	Based on a gene from *Bacillus thuringiensis* subsp. *kumamotoensis*. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes (Schnepf et al., 1998; Ellis et al., 2002)	Event MON 88017 expresses the Cry3Bb1, a protein toxic to certain lepidopteran larvae feeding on maize
59122	Cry34Ab1	Based on a gene from *Bacillus thuringiensis* strain PS149B1. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes (Schnepf et al., 1998; Ellis et al., 2002)	Event 59122 expresses the Cry34Ab1. In complex with Cry35Ab1 this protein is toxic to certain coleopteran larvae feeding on maize
	Cry35Ab1	Based on a gene from *Bacillus thuringiensis* strain PS149B1. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes (Schnepf et al., 1998; Ellis et al., 2002)	Event 59122 expresses the Cry35Ab1. In complex with Cry34Ab1 this protein is toxic to certain coleopteran larvae feeding on maize
	PAT	Based on a gene from *Streptomyces viridochromogenes* Tü494. Phosphinothricin-acetyl-transferase (PAT) enzyme acetylates L-glufosinate-ammonium (Thompson et al., 1987; Wohlleben et al., 1988; Eckes et al., 1989)	Event 59122 expresses the PAT protein which confers tolerance to glufosinate ammonium-based herbicides (Droge-Laser et al., 1994)
DAS-40278-9	AAD-1	Based on a gene from *Sphingobium herbicidovorans*. Aryloxyalkanoate dioxygenase (AAD-1) facilitates the breakdown of phenoxy auxin and aryloxyphenoxypropionate herbicides into carbon sources for the bacterium (Wright et al., 2009)	Event DAS-40278-9 expresses AAD-1 protein which degrades the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and thus confers tolerance to this herbicide

3.3.1.2. Integrity of the events in the five-event stack maize

The genetic stability of the inserted DNA over multiple generations in the single maize events MON 89034, 1507, MON 88017, 59122 and DAS-40278-9 was previously demonstrated (see Table 2). Integrity of these events in the five-event stack maize was demonstrated by Southern analyses.

3.3.1.3. Information on the expression of the inserts

Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT, CP4EPSPS and AAD-1 protein levels were analysed by enzyme-linked immunosorbent assay (ELISA) in material harvested from a field trial across 10 locations in the US in 2010. The five-event stack maize samples analysed included leaf (V2–V4, V9), root (R1), grain (R6), pollen (R1), forage (R4) and whole plant (R6) treated and not treated with intended herbicides. The applicant indicated that a small percentage of the non-GM controls showed detectable levels of the proteins, possibly resulting from cross-contamination or sampling error. Additional information requested by the GMO Panel did not allow limiting this observation to particular locations. Considering that the proportion of contaminated controls was very low and given the high number of samples analysed, the impact on the mean expression values presented in Appendix 1 is considered negligible.

In order to assess changes in protein expression levels which may result from potential interactions between the events, protein levels were determined for the five-event stack maize and one corresponding single events or the already assessed four-event stack maize MON 89034 × 1507 × MON 88017 × DAS-59122-7 (see Table 2) in different parts of the plant grown without intended herbicide regimes.

The levels of the proteins in the five-event stack maize were comparable in all tissues to those of either the single event DAS-40278-9 or the previously assessed four-event stack MON 89034 × 1507 × MON 88017 × DAS-59122-7 (EFSA GMO Panel, 2010c, Appendix 1).Therefore, there is no indication of

interactions that may affect the integrity of the events and the levels of the newly expressed proteins in this stack.

3.3.1.4. Conclusion on molecular characterisation

The molecular data establish that the events stacked in the five-event stack maize have retained their integrity. Protein expression analyses show that the levels of the newly expressed proteins in the five-event stack maize are similar to those of either the single event DAS-40278-9 or the already assessed four-event stack maize MON 89034 × 1507 × MON 88017 × DAS-59122-7. Therefore, there is no indication of an interaction that may affect the integrity of the events and the levels of the newly expressed proteins in this stack.

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

3.3.2. Comparative analysis[7]

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

Application EFSA-GMO-NL-2013-113 presents data on agronomic and phenotypic characteristics, as well as forage and grain composition of the five-event stack maize derived from field trials performed at 10 sites in US during the 2010 growing season (Table 5).

Table 5: Overview of comparative assessment studies with the five-event stack maize

Study focus	Study details	Comparator	Non-GM commercial reference varieties
Agronomic, phenotypic and compositional analysis	Field study, 2010, US, 10 sites	SLB01 × BE9514	Six

GM: genetically modified.

The five-event stack maize was obtained by conventional crossing: events MON 89034, MON 88017 and DAS-40278-9 were introgressed in the inbred line SLB01, while events 1507 and 59122 in BE9514. As documented by the pedigree, the five single events, after backcrossing, were combined in a hybrid maize with a genetic background (F_1) of SLB01 × BE9514. The same two inbred lines (SLB01 and BE9514) were crossed to produce the non-GM hybrid maize used as comparator. On the basis of the provided pedigree, documenting the production of the five-event stack GM maize, the EFSA GMO Panel considers that the hybrid maize SLB01 × BE9514 is a suitable comparator.

The field trial sites were located in major maize growing areas of the US,[8] representing regions of diverse agronomic practices and environmental conditions. At each site, the following materials were grown in a randomised complete block design with four replicates: the five-event stack maize treated, a non-GM comparator and three non-GM reference varieties, all treated (sprayed) with plant protection products (PPP) according to local requirements, and the five-event stack maize treated with the intended herbicides (glyphosate-, glufosinate-ammonium-, quizalofop- and 2,4-D-containing herbicides) in addition to the other PPP.

Statistical analysis of field trials data

The statistical analysis of the agronomic, phenotypic and compositional data from the 2010 field trials followed the recommendations of the GMO Panel (EFSA GMO Panel, 2010b, 2011a). This includes, for each of the two treatments of the five-event stack maize, the application of a difference test (between the GM maize and non-GM comparator) and an equivalence test (between the GM maize and the set of non-GM commercial reference varieties). The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).[9]

[7] Dossier: Part II Scientific information, Section 3.

[8] Richland, Jefferson, Lime Springs and Atlantic in Iowa; Cherry Grove and Geneva in Minnesota; Wyoming, Illinois; Deerfield, Michigan; Brunswick, Nebraska and Germansville, Pennsylvania.

[9] 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).

3.3.2.2. Agronomic and phenotypic analysis

A total of 26 agronomic and phenotypic endpoints, including observations on the biotic and abiotic interactions, were analysed.[10]

Data for 15 endpoints[11] were considered not suitable for a parametric analysis; for these a Wilcoxon signed-rank (WSR) test was used to check for differences between the GM maize and the non-GM comparator.

The remaining 11 endpoints were analysed as described in Section 3.3.2.1, with the following outcomes:

- For the five-event stack maize (not treated with the intended herbicides), statistically significant differences with the non-GM comparator were identified for early population, final population and disease incidence. Early population and final population fell under equivalence category I or II. For disease incidence,[12] the test of equivalence was not applied because the variability among the non-GM reference varieties was estimated to be zero and thus, further assessment was needed.
- For the five-event stack maize (treated with the intended herbicides), statistically significant differences with the non-GM comparator were identified for early population, final population and time to pollen shed. All three endpoints fell under equivalence category I or II.

Of the endpoints analysed with the WSR test, a statistically significant difference was identified for days to maturity. However, the mean difference (in heat units) corresponded to a small fraction of a day.

3.3.2.3. Compositional analysis

Forage and grain harvested from the field trial study in the US in 2010 (Table 5) were analysed for 82 constituents (9 in forage and 73 in grain), including the key constituents recommended by the OECD (OECD, 2002). For 17 grain constituents,[13] more than 50% of the observations were below the limit of quantification. The statistical analysis was applied to the remaining 65 constituents (9 in forage[14] and 56 in grain[15]). A summary of the outcome of the test of difference and the test of equivalence is presented in Table 6:

- For the five-event stack maize (not treated with the intended herbicides), statistically significant differences with the non-GM comparator were identified for 21 grain endpoints and phosphorus in forage. All these endpoints fell under equivalence category I or II, except for grain content of protein, magnesium and manganese which fell under equivalence category III or IV. Levels of leucine, phenylalanine and ferulic acid in grain fell under equivalence category III or IV, although no statistically significant differences were identified with the non-GM comparator.
- For the five-event stack maize (treated with the intended herbicides), statistically significant differences with the non-GM comparator were identified for 31 grain endpoints and 4 forage endpoints. All these endpoints fell under equivalence category I or II, except for levels of protein, glutamic acid, glycine, leucine, lysine, threonine, magnesium and manganese in grain, which fell under equivalence category III or IV. Levels of cystine, phenylalanine and iron in grain fell under equivalence category III or IV, although no statistically significant differences were identified with the non-GM comparator.

[10] Early population, final population, time to silking, time to pollen shed, plant height, ear height, yield, pollen colour (measured at 0, 30, 60 and 120 min), pollen shape (measured at 0, 30, 60 and 120 min), stay green, herbicide injury (after each of 4 herbicide applications), disease incidence, insect damage, days to maturity, root lodging, stalk lodging and seedling vigour.

[11] Days to maturity, herbicide injury (at 4 time points), insect damage, pollen colour (at 0, 60 and 120 min), pollen shape (at 0, 60 and 120 min), root lodging, seedling vigour, and stalk lodging.

[12] Estimated mean values for disease incidence (% plant tissue/leaf area with symptoms) were the following: 15.58% (non-GM comparator); 19.02% (untreated GM maize); and 18.74% (non-GM commercial reference varieties).

[13] Sodium, furfural, ascorbic acid and the fatty acids caprylic (8:0), capric (10:0), lauric (12:0), myristic (14:0), myristoleic (14:1), pentadecanoic (15:0), pentadecenoic (15:1), palmitoleic (16:1), heptadecanoic (17:0), heptadecenoic (17:1), γ-linolenic (18:3), eicosadienoic (20:2), eicosatrienoic (20:3) and arachidonic (20:4).

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

[15] Proximates (protein, fat, ash, moisture and carbohydrates by calculation), fibre fractions (acid detergent fibre (ADF), neutral detergent fibre (NDF) and total detergent fibre (TDF)), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, selenium and zinc), amino acids (alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine), fatty acids ((palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), arachidic acid (20:0), eicosenoic acid (20:1) and behenic acid (22:0)), vitamins (β-carotene, thiamine, riboflavin, niacin, pyridoxine, folic acid and α-tocopherol) and other compounds (inositol, *p*-coumaric acid, ferulic acid, phytic acid, raffinose and trypsin inhibitor).

Table 6: Outcome of the comparative compositional analysis in grains and forage of the five-stack maize MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9. The table shows the number of endpoints in each category

		Test of difference[a]			
		Not treated[c]		Treated[c]	
		Not different	Significantly different	Not different	Significantly different
Test of equivalence[b]	Category I/II	38	19[d]	25	27[d]
	Category III/IV	3[e]	3[f]	3[e]	8[f]
	Not categorised	2[g]	–	2[g]	–
	Total endpoints	65		65	

(a): Comparison between maize MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9 and its non-GM comparator.
(b): Four different outcomes: 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). Not categorised means that the test of equivalence was not applied because of the lack of variation among the non-GM reference varieties.
(c): Treated/not-treated with the intended herbicides (see Section 3.3.2.1).
(d): Endpoints with significant differences between maize MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9 and its non-GM comparator falling in equivalence category I-II (treated and not treated).
For grain, both treated and not treated: p-coumaric acid, inositol, phytic acid, palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), ash, total fat, phosphorus, potassium, zinc, beta-carotene (A), thiamine (B1) and alpha-tocopherol. For not treated only: selenium and riboflavin (B2). For treated only: arginine, histidine, valine, eicosenoic acid (20:1), ferulic acid, moisture and carbohydrates.
For forage, both treated and not treated: phosphorus. For treated only: ash, carbohydrates and protein.
(e): The following endpoints in grain fell under equivalence category III or IV, although no statistically significant differences were identified with respect to the comparator: phenylalanine (both treated and not treated), leucine and ferulic acid (not treated only), cystine and iron (treated only).
(f): Endpoints with significant differences between maize MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9 and its non-GM comparator and falling in equivalence category III–IV. Quantitative results for these endpoints are reported in Table 7.
(g): Endpoints not categorised for equivalence and with no significant differences between maize MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9 (both treated and not treated) and its non-GM comparator: isoleucine and trypsin inhibitor in grain.

The GMO Panel assessed all significant differences between the five-event stack maize and the non-GM comparator, taking into account potential impact on plant metabolism and the natural variability observed for the set of non-GM commercial reference varieties. Quantitative results for the endpoints showing significant differences between the five-event stack maize and the non-GM comparator and falling under category III/IV are given in Table 7.

Table 7: Quantitative results (estimated means and equivalence limits) for compositional endpoints in grain that are further assessed based on the results of the statistical analysis

Endpoint	Maize MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9		Non-GM comparator	Non-GM reference varieties	
	Not treated	Treated[a]		Mean	Equivalence limits
Glutamic acid (%AA)	18.98	19.08*	18.91	18.48	(17.98, 19.00)
Glycine (%AA)	3.746	3.681*	3.776	4.026	(3.693, 4.391)
Leucine (%AA)	12.70	12.84*	12.63	12.09	(11.55, 12.67)
Lysine (%AA)	2.720	2.646*	2.772	2.967	(2.649, 3.326)
Threonine (%AA)	3.553	3.516*	3.558	3.64	(3.531, 3.752)
Protein (% dw)	11.40*	11.06*	10.73	9.53	(8.16, 10.89)
Magnesium (mg/100 g dw)	136.5*	136.6*	126.4	110.1	(89.9, 134.7)

Endpoint	Maize MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9		Non-GM comparator	Non-GM reference varieties	
	Not treated	Treated[(a)]		Mean	Equivalence limits
Manganese (mg/100 g dw)	0.788*	0.834*	0.748	0.550	(0.402, 0.754)

GM: genetically modified; dw: dry weight; % AA: percentage total amino acid.
(a): Treated with the intended herbicides as described in Section 3.3.2.1.
For the GM maize, significantly different entries are marked with an asterisk, while the outcomes of the test of equivalence are differentiated by a greyscale background: white (equivalence category I or II), light grey (equivalence category III) and dark grey (equivalence category IV).

3.3.2.4. Conclusion on comparative analysis

Based on the agronomic and phenotypic characteristics of the five-event stack maize tested under field conditions, none of the differences observed between the five-event stack maize its non-GM comparator are further assessed for potential environmental impact, except for the endpoint disease incidence which is further assessed in Section 3.3.4.1.

The GMO Panel also concludes that none of the differences identified in forage and grain composition between the five-event stack maize, the non-GM comparator and the non-GM commercial reference varieties needs further assessment regarding food and feed safety, except for the changes in levels of protein, glutamic acid, glycine, leucine, lysine, threonine, magnesium and manganese in grain, which are further assessed in Section 3.3.3.

3.3.3. Food and feed safety assessment[16]

3.3.3.1. Effects of processing

The five-event stack maize will undergo existing production processes used for conventional maize. Based on the outcome of the comparative assessment, processing of the five-event stack maize into food and feed products is not expected to result in products being different from those of non-GM maize varieties.

3.3.3.2. Influence of Temperature and pH on newly expressed proteins

Effects of temperature and pH on Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT, CP4 EPSPS and AAD-1 proteins have been previously evaluated by the GMO Panel (Table 2). In the context of this application, no new studies addressing these aspects were provided by the applicant.

3.3.3.3. Toxicology

Testing of newly expressed proteins

Nine proteins (Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT, CP4 EPSPS and AAD-1) are newly expressed in the five-event stack maize (see Section 3.1). The GMO Panel has previously assessed these proteins in the context of the single maize events (see Table 2), and no safety concerns were identified for humans and animals. The GMO Panel is not aware of any new information that would change these conclusions.

The potential for a functional interaction between the proteins newly expressed in the five-event stack maize has been assessed with regard to human and animal health. The CP4 EPSPS, PAT and AAD-1 proteins are enzymes that catalyse distinct biochemical reactions and act on unrelated substrates with high substrate specificity. The Cry1A.105, Cry2Ab2, Cry3Bb1, Cry3Bb1, Cry34Ab1 and Cry1F proteins are delta endotoxins with highly specific insecticidal properties acting through cellular receptors found in target insect species. It is reported that the gastrointestinal tract of mammals, including humans, lacks receptors with high affinity to Cry proteins (Hammond et al., 2013; Koch et al., 2015). On the basis of the known biological functions of the individual newly expressed proteins (Table 4), there is currently no expectation for possible interactions relevant for the food and feed safety of the five-event stack maize.

In vitro protein degradation studies on Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT, CP4 EPSPS and AAD-1 proteins have been previously evaluated by the EFSA GMO Panel (Table 2).

[16] Dossier: Part II Scientific information, Sections 4, 5 and 6.

In the context of this application, no new studies addressing *in vitro* protein degradation of these newly expressed proteins were provided by the applicant.

The GMO Panel concludes that there are no safety concerns for human and animal health related to the newly expressed proteins Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT, CP4 EPSPS and AAD-1 in the five-event stack maize.

Testing of new constituents other than newly expressed proteins

No new constituents other than newly expressed proteins have been identified in the five-event stack maize. Therefore, no further food and feed safety assessment of components other than the newly expressed proteins is required.

Information on altered levels of food and feed constituents

Protein, glutamic acid, glycine, leucine, lysine, threonine, magnesium and manganese in grain were significantly different in the five-event stack maize when compared to its comparator and showed lack of equivalence with the set of non-GM reference varieties (see Section 3.3.2.3). Taking into account the known biological role of these compounds, these differences are considered of no toxicological concern by the GMO Panel. Further information on the safety of these maize constituents is provided in Section 3.3.3.5.

Testing of the whole genetically modified food and feed

Based on the outcome of the studies considered in the molecular characterisation and comparative analysis, no substantial modifications of toxicological relevance in the composition of the five-event stack maize, and no indication of possible unintended effects relevant to food and feed safety have been identified (see Sections 3.3.1 and 3.3.2.3). Therefore, animal studies on food and feed from the five-event stack maize are not necessary (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 protein

For allergenicity, the GMO Panel has previously evaluated the safety of Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT, CP4 EPSPS and AAD-1 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 are expected.

For adjuvanticity, Cry1Ac protein has been suggested to possess adjuvant activity based on animal studies 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 evaluated in the four-event stack MON 89034 × 1507 × MON 88017 × DAS-59122-7 (see Appendix A). From the limited evidence available, the GMO Panel did not find indications that the presence of the Cry 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 the whole GM plant

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

In the context of this application and considering the data from the molecular characterisation, the compositional analysis and the assessment of the newly expressed proteins (see Sections 3.3.1, 3.3.2 and 3.3.3), the GMO Panel identifies no indications of a potentially increased allergenicity of foods and feeds from the five-event stack maize with respect to those from its non-GM comparator.

3.3.3.5. Nutritional assessment of GM food and feed

The intended trait of the five-event stack maize is insect resistance and herbicide tolerance, with no intention to alter the nutritional parameters. However, levels of protein, glutamic acid, glycine, leucine, lysine, threonine, magnesium and manganese in grains from the five-event stack maize were significantly different from its non-GM comparator and showed a lack of equivalence with the set of non-GM reference varieties (Section 3.3.2.3). The biological role of these compounds, the contribution of maize to their total intake and the magnitude and direction of the observed changes are considered in the nutritional assessment.

Human nutrition

A relatively small increase of protein content (3–6% as compared to its non-GM comparator) was observed in the GM-maize what does not imply any concern from nutritional point of view. Maize protein is considered of low nutritional quality due to a poor balance of essential amino acids, in particular due to the low levels of lysine and tryptophan. Among the five amino acids where significant differences were observed as compared to its non-GM comparator, two of them are essential amino acids: lysine and threonine. A decrease of approximately 1% and 5% in their relative amounts (expressed as percentage of total amino acids) was observed for threonine and lysine, respectively. Lysine is already a limiting amino acid in conventional maize while the amount of threonine present in maize protein represents 157% of the requirements of this essential amino acid (FAO/WHO/UNU, 2007). Although this implies that the GM maize protein might be slightly poorer in amino acid composition than the conventional one, the higher amount of protein in the GM maize indicates a similar intake of these essential amino acids per amount of maize consumed. Therefore, the nutritional impact of GM maize is considered the same as that of the non-GM comparator.

Increases up to 8% in manganese and up to 11% in magnesium were observed in the GM maize as compared to its non-GM comparator. These two minerals are essential elements for humans and Adequate Intakes (AI) have been proposed (EFSA, 2017a,b). While no Tolerable Upper Intake Levels (UL) are set for manganese, for magnesium the UL refer to dissociable Mg salts (e.g. chloride, sulfate, aspartate, lactate) and compounds like MgO in food supplements, in water or added to foods. However, this UL does not include magnesium normally present in foods and beverages. In maize, several minerals including manganese and magnesium are usually bound to phytic acid what decreases substantially their bioavailability (Gupta et al., 2015; Suri and Tanumihardjo, 2016). Therefore, the increase observed in these two essential elements does not represent any concern from nutritional point of view.

Animal nutrition

Glutamic acid and glycine are not essential amino acids; the magnitude of their respective increase and decrease in maize grains does not pose an issue for animal nutrition. Leucine, lysine and threonine are essential amino acids; the increase observed in leucine in maize grains is not a problem for animal nutrition. The magnitude of the decrease in lysine and threonine in maize grains does not pose a problem for animal nutrition; these amino acids are usually balanced and supplemented in the complete diets (e.g. monogastric animals). Maize grains are not considered a major source of proteins in animals and the increase observed does not pose an issue for animals; it improves the protein energy ratio in maize.

[17] 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.

Among minerals, magnesium and manganese are respectively considered major and trace elements important in animal nutrition; the observed changes do not pose an issue for animals, since complete diets are balanced with mineral premixes. Moreover, maize grains are also considered a poor source of manganese (McDonald et al., 2011).

Conclusion on human and animal nutrition

Based on the current knowledge on the biological role of the compounds assessed, the magnitude and direction of the changes identified, and the relevance of maize as contributor to the intake of these compounds, the GMO Panel concludes that the nutritional impact of foods and feeds from the five-event stack maize is expected to be the same as those from the comparator and non-GM reference varieties.

3.3.3.6. Conclusion on food and feed safety assessment

The individual proteins Cry1A.105, Cry2Ab2, PAT, Cry1F, Cry3Bb1, CP4-EPSPS, Cry34Ab1, Cry35Ab1 and AAD-1 newly expressed in the five-event stack maize do not raise safety concerns for human and animal health. Interactions between these newly expressed proteins raising food and feed safety concerns (toxicological, allergenicity and adjuvanticity) are not expected. The nutritional impact of the five-event stack maize foods and feeds is expected to be the same as those from the non-GM comparator and non-GM reference varieties. The GMO Panel concludes that the five-event stack maize, as described in this application, is as safe as and nutritionally equivalent to the non-GM comparator and the non-GM reference varieties tested.

3.3.4. Environmental risk assessment[18]

Considering the scope of application EFSA-GMO-NL-2013-113, which excludes cultivation, the environmental risk assessment (ERA) of the five-event stack maize mainly takes into account: (1) the exposure of microorganisms to recombinant DNA in the gastrointestinal tract of animals fed GM material and of microorganisms present in environments exposed to faecal material of these animals (manure and faeces); and (2) the accidental release into the environment of viable five-event stack maize grains during transportation and/or processing (EFSA GMO Panel, 2010a).

3.3.4.1. Persistence and invasiveness of the GM plant

Maize is highly domesticated, not winter hardy in colder regions of Europe, and generally unable to survive in the environment without appropriate management. Occasional feral GM maize plants may occur outside cultivation areas in the EU (e.g. Pascher, 2016), but survival is limited mainly by a combination of low competitiveness, the absence of a dormancy phase and susceptibility to plant pathogens, herbivores and cold climate conditions (OECD, 2003). Field observations indicate that maize grains may survive and overwinter in some EU regions, resulting in volunteers in subsequent crops (e.g. Gruber et al., 2008; Palaudelmàs et al., 2009; Pascher, 2016). However, maize volunteers have been shown to grow weakly and flower asynchronously with the maize crop (Palaudelmàs et al., 2009). Thus, the establishment and survival of feral and volunteer maize in the EU is currently limited and transient.

It is unlikely that the intended traits of the five-event stack maize and the observed difference in disease incidence (see Section 3.3.2.2) will provide a selective advantage to maize plants, except when they are exposed to glyphosate-, glufosinate-ammonium-, 2,4-D-containing herbicides or certain AOPP herbicides (such as quizalofop), or infested by insect pests that are susceptible to the Cry1A.105, Cry1F, Cry2Ab2, Cry34Ab1, Cry35Ab1 or Cry3Bb1 proteins. However, this fitness advantage will not allow the five-event stack maize to overcome other biological and abiotic factors (described above) limiting plant's persistence and invasiveness. Therefore, the presence of the intended traits will not affect the persistence and invasiveness of the GM plant.

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

[18] Dossier: Part II Scientific information, Sections E3.1, E.3.2., D 9.4., D 9.5., D 9.8.

3.3.4.2. Potential for gene transfer

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 probability and potential adverse effects of HGT of the recombinant DNA have been assessed in previous GMO Panel scientific opinions for the single events (see Table 2). No concern as a result of an unlikely, but theoretically possible, HGT of the recombinant genes to bacteria in the gut of domesticated animals and humans fed GM material or other receiving environments was identified. The applicant submitted an updated bioinformatic analysis for each of the single events in order to assess the possibility for HGT by homologous recombination.

The updated bioinformatic analysis for maize event 1507 revealed sufficient length and sequence identity for homologous recombination for two copies of the ORF25 terminator with the same *A. tumefaciens* genomic sequence. Because of its length (~ 700 bp) and the opposite orientation of the two ORF25 copies in maize event 1507, a potential for a facilitated HGT by double homologous recombination (DHR) is unlikely. The occurrence of a DHR would result in the insertion of the *pat* gene cassette which is expected to be less efficiently translated in potential bacterial recipients because of the plant-codon optimisation of the *pat* gene and because the *pat* gene is under the control of plant virus element.

The updated bioinformatic analyses for maize events MON 89034, MON 88017 and 59122 do not reveal any new DNA sequence that could provide sufficient length and identity which could facilitate HGT by DHR, confirming the previous conclusions (EFSA GMO Panel, 2017b,c).

Bioinformatic analysis for maize event DAS-40278-9 does not reveal sufficient length and sequence identity with known sequences from bacteria which would facilitate homologous recombination.

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.

Therefore, the GMO Panel concludes that the unlikely, but theoretically possible, horizontal transfer of recombinant genes from this five-event stack maize to bacteria does not raise any environmental safety concern.

Plant-to-plant gene transfer

The potential for occasional feral five-event stack maize plants originating from grain import spills to transfer recombinant DNA to sexually compatible plants and the environmental consequences of this transfer were considered.

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

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

The potential of spilled maize grains to establish, grow and produce pollen is extremely low and transient (see Section 3.3.4.1). Therefore, the likelihood/frequency of cross-pollination between occasional feral GM maize plants resulting from grain spillage, and weedy or cultivated *Zea* plants is considered extremely low (EFSA, 2016). Even if cross-pollination would occur, the GMO Panel is of the opinion that environmental effects as a consequence of the spread of genes from occasional feral GM maize plants in Europe will not differ from that of conventional maize varieties for the reasons given in Section 3.3.4.1, even if exposed to the intended herbicides.

3.3.4.3. Interactions of the GM plant with target organisms

Taking the scope of application EFSA-GMO-NL-2013-113 into account (no cultivation), potential interactions of occasional feral the five-event stack maize plants arising from grain import spills with target organisms are not considered a relevant issue.

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

Given that environmental exposure of non-target organisms to spilled GM grains or occasional feral GM maize plants arising from spilled five-event stack maize grains is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions of the five-event stack maize with non-target organisms are not considered to raise any environmental safety concern. Interactions that may occur between the Cry proteins would not alter this conclusion.

3.3.4.5. Interactions with abiotic environment and biogeochemical cycles

Given that environmental exposure to spilled grains or occasional feral five-event stack maize plants arising from grain import spills is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions with the abiotic environment and biogeochemical cycles are not considered to raise any environmental safety concern.

3.3.4.6. Conclusion on environmental risk assessment

The GMO Panel concludes that it is unlikely that the five-event stack maize would differ from conventional maize varieties in its ability to persist under EU environmental conditions. Considering the scope of application EFSA-GMO-NL-2013-113, interactions of occasional feral five-event stack maize plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from the five-event stack maize to bacteria does not indicate a safety concern. Therefore, considering the combined traits and their interactions, the outcome of the comparative analysis, and the routes and levels of exposure, the GMO Panel concludes that the five-event stack maize 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 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9

No new data on the single maize events MON 89034, 1507, MON 88017, 59122 and DAS-40278-9 leading to a modification of the original conclusions on their safety are identified.

The molecular characterisation, comparative analysis (agronomic, phenotypic and compositional characteristics) and the outcome of the toxicological, allergenicity and nutritional assessment indicates that the combination of the single maize events and of the newly expressed proteins in the five-event stack maize does not give rise to food and feed safety and nutritional concerns. The GMO Panel concludes that the five-event stack maize, as described in this application, is as safe as and nutritionally equivalent to its non-GM comparator and the non-GM reference varieties tested.

Considering the combined traits and their interactions, the outcome of the comparative analysis, and routes and levels of exposure, the GMO Panel concludes that the five-event stack maize would not raise safety concerns in the event of accidental release of viable GM maize grains into the environment.

No scientific information that could change the conclusions on this five-event stack was retrieved in a literature search covering the period since the time of validity of the application.[19]

In conclusion, the GMO Panel considers that the five-event stack maize is as safe as its non-GM comparator and the tested non-GM maize reference varieties with respect to potential effects on human and animal health and the environment.

3.4. Risk assessment of the subcombinations[20]

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

The strategy followed for the assessment of those subcombinations for which no specific data have been submitted and which have not been previously assessed by the GMO Panel (see Table 8), has been described by the GMO Panel.[21] In this case, the risk assessment takes as its starting point the assessment of the single maize events, and uses the data generated for the five-event stack, as well

[19] Additional information: 22/12/2017.
[20] Additional information: 22/3/2018, 18/6/2018.
[21] 115th GMO Panel meeting (Annex 1 of the minutes: https://www.efsa.europa.eu/sites/default/files/event/170517-m.pdf).

as all the additional data available on subcombinations previously assessed by the GMO Panel (see Table 2).

3.4.1. Subcombinations previously assessed

The GMO Panel has previously assessed 11 subcombinations (six-two-event stacks, four-three-event stacks and one-four-event stack, see Table 2) and did not identify any safety concerns. No scientific information relevant to the risk assessment of these maize stacks became available since the validation of application EFSA-GMO-NL-2013-113. Consequently, the GMO Panel considers that its previous conclusions on these subcombinations remain valid.

3.4.2. Subcombinations not previously assessed

Fourteen 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 8).

Table 8: Subcombinations of maize MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9 not previously assessed and covered by the scope of application EFSA-GMO-NL-2013-113

Degree of stacking	Events
Four-event stack maize	MON 89034 × 1507 × MON 88017 × DAS-40278-9
	MON 89034 × 1507 × 59122 × DAS-40278-9
	MON 89034 × MON 88017 × 59122 × DAS-40278-9
	1507 × MON 88017 × 59122 × DAS-40278-9
Three-event stack maize	MON-89034 × MON 88017 × DAS-40278-9
	MON-89034 × 59122 × DAS-40278-9
	MON 89034 × 1507 × DAS-40278-9[(a)]
	1507 × MON 88017 × DAS-40278-9
	1507 × 59122 × DAS-40278-9
	MON 88017 × 59122 × DAS-40278-9
Two-event stack maize	MON 88017 × DAS-40278-9
	MON-89034 × DAS-40278-9[(a)]
	1507 × DAS-40278-9[(a)]
	59122 × DAS-40278-9

(a): Subcombinations assessed in parallel in the context of application EFSA-GMO-NL-2013-112 (EFSA GMO Panel, 2019).

3.4.2.1. Stability of the events

The genetic stability of the inserted DNA over multiple generations in the five single maize events has been demonstrated previously (see Table 2). Integrity of the events has been demonstrated in the five-event stack maize (Section 3.3.1.2) and the previously assessed maize subcombinations (see Table 2). The GMO Panel finds no reasons to expect loss of integrity of the events in the maize subcombinations not previously assessed (see Table 8).

3.4.2.2. Expression of the events

The GMO Panel assessed whether any 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 is further supported in previous GMO Panel assessments for instance in two-event maize stack MON89034 x MON88017 where it was concluded that the expression levels of Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS proteins in the stacked line were comparable to those in the single events (EFSA, 2010). Similar conclusion was drawn for proteins Cry34Ab1, Cry35Ab1, Cry1F and PAT when assessing the two-event maize stack 1507 × 59122 (EFSA, 2009c). Furthermore, in the assessment of the four-event maize stack MON 89034 × 1507 × MON 88017 × 59122, the GMO Panel concluded that the expression levels of proteins Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, CP4

EPSPS, Cry34Ab1 and Cry35Ab1 are comparable to those in the single events (EFSA GMO Panel, 2010c). 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-NL-2013-113.

3.4.2.3. Potential functional interactions between the events

The GMO Panel assessed the potential for interactions between maize events in the subcombinations not previously assessed (Table 8), 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 relevant for the food and feed or environmental safety between these proteins in the 14 subcombinations not previously assessed. 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. It is concluded that none of these effects would raise safety concerns when combined in any of these maize subcombinations. Therefore, the GMO Panel is of the opinion that no additional data are needed to complete the assessment of subcombinations from the five-event stack maize.

3.4.3. Conclusion

Since no new safety concerns were identified for the previously assessed 11 subcombinations (six-two-event stacks, four-three-event stacks and one-four-event stack), 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-NL-2013-113 for which no experimental data have been provided, the GMO Panel has assessed the possibility of interactions between the events and concludes that these combinations would not raise safety concerns. These subcombinations are therefore expected to be as safe as and nutritionally equivalent to the single maize events, the previously assessed 11 subcombinations as well as the five-event stack maize.

3.5. Post-market monitoring[22]

3.5.1. Post-market monitoring of GM food/feed

The GMO Panel concludes that the five-event stack maize, as described in this application, is nutritionally equivalent to and as safe as the non-GM comparator and the non-GM reference varieties tested, and no post-market monitoring (EFSA GMO Panel, 2011a) of food and feed is considered necessary.

Eleven subcombinations (six-two-event stacks, four-three-event stacks and one-four-event stack, see Table 2) 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-NL-2013-13 are expected to be as safe as and nutritionally equivalent to the single maize events, the previously assessed maize subcombinations and the five-event stack maize. Therefore, the GMO Panel considers that post-market monitoring of the five-event stack maize and its subcombinations, as described in this application, is not necessary.

3.5.2. Post-market environmental monitoring

The objectives of a post-market environmental monitoring (PMEM) plan, according to Annex VII of Directive 2001/18/EC, are: (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the ERA are correct; and (2) to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment that were not anticipated in the ERA.

Monitoring is 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 rationale 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.

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

The PMEM plan proposed by the applicant for the five-event stack maize and its subcombinations 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 intended uses of the five-event stack maize. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan. The PMEM plan and reporting intervals are in line with the intended uses of the five-event stack maize and its subcombinations.

3.5.3. Conclusion on post-market monitoring

No post-market for food and feed is necessary. The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of the five-event stack maize and its subcombinations.

4. Overall conclusions and recommendations

No new data on the five single maize events MON 89034, 1507, MON 88017, 59122 and DAS-40278-9 that would lead to a modification of the original conclusions on their safety are identified.

The molecular characterisation, comparative analysis (agronomic, phenotypic and compositional characteristics) and the outcome of the toxicological, allergenicity and nutritional assessment indicate that the combination of the single maize events and of the newly expressed proteins in the five-event stack maize does not give rise to food and feed safety and nutritional concerns. The GMO Panel concludes that the five-event stack maize, as described in this application, is as safe as and nutritionally equivalent to its non-GM comparator and the non-GM reference varieties tested, and no post-market monitoring of food and feed is considered necessary.

The GMO Panel concludes that there is a very low likelihood of environmental effects resulting from the accidental release of viable grains from five-event stack maize into the environment.

Since no new data on the 11 subcombinations (six-two-event stacks, four-three-event stacks and one-four-event stack) previously assessed that would lead to a modification of the original conclusions on their safety are 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-NL-2013-113 for which no experimental data have been provided, the GMO Panel assessed possible interactions between the events, and concludes that combinations of maize events MON 89034, 1507, MON 88017, 59122 and DAS-40278-9 would not raise safety concerns in these maize subcombinations. These subcombinations are therefore expected to be as safe as and nutritionally equivalent to the maize single events, the previously assessed subcombinations and the five-event stack maize.

Given the absence of safety concerns for foods and feeds from the five-event stack maize and all its subcombinations, the GMO Panel considers that post-market monitoring of these products is not necessary.

The PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of the five-event stack maize and its subcombinations.

Documentation provided to EFSA

1) Application EFSA-GMO-NL-2013-113 received from the Competent Authority of Netherlands in support to Dow AgroSciences request for placing maize MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9 on the EU market according to Regulation (EC) No 1829/2003, 6 February 2013.
2) Receipt of application EFSA-GMO-NL-2013-113 acknowledged by EFSA, 28 February 2013.
3) Application EFSA-GMO-NL-2013-113 validated by EFSA, 2 October 2014.
4) Receipt of spontaneous information from the applicant, 24 April 2015.
5) Request for supplementary information to the applicant, 11 August 2016.
6) Receipt of supplementary information from the applicant, 13 October 2016.

7) Request for supplementary information to the applicant, 3 November 2016.
8) Request for supplementary information to the applicant, 9 November 2016.
9) Request for supplementary information to the applicant, 21 December 2016.
10) Receipt of supplementary information from the applicant, 24 January 2017.
11) Request for supplementary information to the applicant, 27 January 2017.
12) Receipt of supplementary information from the applicant, 7 February 2017.
13) Receipt of supplementary information from the applicant, 6 March 2017.
14) Request for supplementary information to the applicant, 14 March 2017.
15) Request for supplementary information to the applicant, 11 April 2017.
16) Request for supplementary information to the applicant, 18 May 2017.
17) Receipt of supplementary information from the applicant, 8 June 2017.
18) Receipt of supplementary information from the applicant, 19 September 2017.
19) Request for supplementary information to the applicant, 25 September 2017.
20) Receipt of supplementary information from the applicant, 23 November 2017.
21) Receipt of supplementary information from the applicant, 22 December 2017.
22) Request for supplementary information to the applicant, 17 January 2018.
23) Receipt of supplementary information from the applicant, 18 January 2018.
24) Receipt of supplementary information from the applicant, 12 March 2018.
25) Request for supplementary information to the applicant, 22 March 2018.
26) Request for supplementary information to the applicant, 4 May 2018.
27) Receipt of supplementary information from the applicant, 7 May 2018.
28) Request for supplementary information to the applicant, 18 June 2018.
29) Request for supplementary information to the applicant, 29 August 2018.
30) Receipt of supplementary information from the applicant, 20 September 2018.
31) Receipt of supplementary information from the applicant, 10 October 2018.
32) Receipt of supplementary information from the applicant, 20 November 2018.

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Abbreviations

2,4-D	2,4-dichlorophenoxyacetic acid
AAD-1	aryloxyalkanoate dioxygenase 1
ADF	acid detergent fibre
AI	Adequate Intakes
AOPP	aryloxyphenoxypropionate
CTP	chloroplast transit peptide
cry	crystal protein
DHR	double homologous recombination
dw	dry weight
ELISA	enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	environmental risk assessment
GM	genetically modified
GMO	genetically modified organism
GMO Panel	EFSA Panel on Genetically Modified Organisms
HGT	horizontal gene transfer
IgE	immunoglobulin E
NDF	neutral detergent fibre

OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PAT	phosphinothricin acetyltransferase
PMEM	post-market environmental monitoring
PPP	plant protection products
TDF	total detergent fibre
UL	tolerable upper intake levels
UTR	untranslated region
WSR	Wilcoxon signed-rank

Appendix A – Protein expression data

Mean, standard deviation and range of protein levels (μg/g dry weight) from maize MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9, MON 89034 × 1507 × MON 88017 × DAS-59122-7, and DAS-402789 unsprayed tissues from field trials performed across 10 locations in the US in 2010 (n = 40)

	DAS-40278-9	MON 89034 × 1507 × MON 88017 × DAS-59122-7	MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9
Cry1A.105			
Leaf V2–V4		196.56 ± 64.09 (104.14–296.10)	190.28 ± 70.49 (80.50–284.70)
Leaf V9		56.85 ± 24.76 (33.30–118.81)	65.39 ± 25.93 (37.49–123.81)
Leaf R1		38.43 ± 14.50 (23.14–65.61)	40.38 ± 16.25 (20.45–75.65)
Root R1		17.22 ± 4.83 (10.01–24.32)	19.47 ± 5.59 (11.69–25.86)
Forage R4		27.24 ± 10.42 (10.03–45.78)	22.90 ± 6.71 (14.47–30.79)
Whole plant R6		8.90 ± 3.47 (5.08–16.09)	7.55 ± 2.89 (4.66–14.69)
Pollen R1		17.10 ± 2.01 (12.96–20.18)	17.08 ± 1.20 (14.63–18.49)
Grain R6		5.62 ± 0.74 (4.44–6.73)	4.96 ± 0.77 (4.15–6.56)
Cry2Ab2			
Leaf V2–V4		161.39 ± 35.06 (108.60–211.50)	150.88 ± 56.61 (90.00–279.67)
Leaf V9		70.19 ± 16.98 (44.58–104.64)	69.68 ± 18.23 (43.23–107.05)
Leaf R1		64.22 ± 28.77 (25.00–115.23)	64.73 ± 26.66 (24.18–120.09)
Root R1		36.35 ± 12.57 (18.82–53.25)	40.43 ± 17.67 (20.62–69.29)
Forage R4		39.80 ± 9.42 (29.45–52.33)	38.52 ± 9.55 (25.25–51.10)
Whole plant R6		22.72 ± 12.56 (7.74–47.68)	20.67 ± 9.82 (7.43–38.79)
Pollen R1		0.52 ± 0.11 (0.37–0.71)	0.54 ± 0.14 (0.36–0.78)
Grain R6		2.66 ± 0.40 (2.15–3.35)	2.73 ± 0.67 (1.59–3.44)
Cry1F			
Leaf V2–V4		21.29 ± 8.43 (12.30–35.94)	21.74 ± 10.30 (11.28–40.00)
Leaf V9		7.54 ± 2.42 (5.28–12.59)	8.97 ± 2.89 (5.73–14.06)
Leaf R1		7.38 ± 3.25 (4.44–13.30)	8.12 ± 3.50 (3.75–15.70)
Root R1		4.40 ± 0.88 (3.05–5.85)	4.80 ± 1.06 (3.63–6.47)
Forage R4		6.63 ± 1.75 (4.17–10.58)	6.51 ± 1.27 (5.23–9.47)
Whole plant R6		3.72 ± 0.90 (2.51–5.14)	3.45 ± 0.66 (2.61–4.77)
Pollen R1		14.43 ± 1.72 (12.32–17.47)	13.97 ± 1.63 (12.11–16.45)
Grain R6		2.43 ± 0.16 (2.13–2.76)	2.38 ± 0.28 (2.01–3.00)
Cry3Bb1			
Leaf V2–V4		225.30 ± 48.09 (170.27–306.50)	226.79 ± 53.56 (152.13–312.50)
Leaf V9		112.25 ± 18.58 (87.05–140.70)	108.31 ± 16.51 (75.15–127.30)
Leaf R1		104.86 ± 41.49 (60.55–176.25)	95.83 ± 34.92 (53.75–165.85)
Root R1		67.82 ± 15.83 (47.95–88.50)	68.54 ± 17.44 (44.89–92.55)
Forage R4		66.44 ± 8.96 (54.23–81.60)	63.55 ± 8.02 (49.48–77.93)
Whole plant R6		23.05 ± 6.88 (11.03–30.57)	20.88 ± 5.87 (9.01–28.34)
Pollen R1		9.29 ± 1.18 (7.26–10.63)	9.15 ± 0.97 (8.05–10.91)
Grain R6		6.13 ± 0.81 (4.83–7.30)	5.88 ± 0.47 (5.30–6.71)
Cry34Ab1			
Leaf V2–V4		86.31 ± 28.52 (47.30–147.47)	81.82 ± 26.84 (39.40–124.20)
Leaf V9		75.27 ± 20.22 (39.50–105.38)	75.13 ± 19.50 (39.50–102.20)
Leaf R1		116.96 ± 35.80 (75.00–176.69)	127.57 ± 46.75 (75.75–211.65)
Root R1		62.93 ± 18.81 (42.10–90.93)	64.18 ± 24.00 (34.78–94.01)
Forage R4		128.20 ± 30.34 (78.50–190.77)	124.10 ± 22.79 (96.85–166.79)
Whole plant R6		99.08 ± 30.72 (64.50–149.26)	92.78 ± 28.06 (58.10–130.00)
Pollen R1		78.12 ± 27.93 (46.65–142.07)	74.60 ± 27.70 (50.30–138.06)

	DAS-40278-9	MON 89034 × 1507 × MON 88017 × DAS-59122-7	MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9
Grain R6		44.04 ± 7.18 (33.20–56.20)	41.30 ± 5.94 (30.07–48.70)
Cry35Ab1			
Leaf V2–V4		33.50 ± 9.80 (22.77–52.90)	32.24 ± 8.13 (21.37–46.35)
Leaf V9		37.36 ± 14.20 (21.56–60.76)	38.50 ± 12.82 (23.31–62.35)
Leaf R1		51.05 ± 14.44 (37.00–88.45)	50.82 ± 11.82 (36.45–75.00)
Root R1		7.19 ± 1.59 (4.87–9.71)	6.93 ± 1.94 (4.48–9.93)
Forage R4		17.64 ± 2.41 (14.50–22.21)	16.98 ± 2.23 (13.13–19.50)
Whole plant R6		9.26 ± 4.72 (4.03–17.95)	8.35 ± 4.57 (3.83–18.26)
Pollen R1		ND ± NA (ND–0.14)	ND ± NA (ND–(0.04))
Grain R6		0.92 ± 0.16 (0.62–1.14)	0.95 ± 0.18 (0.73–1.21)
CP4 EPSPS			
Leaf V2–V4		148.51 ± 40.95 (80.55–218.70)	148.96 ± 59.33 (69.75–253.13)
Leaf V9		92.50 ± 22.39 (57.40–118.20)	108.24 ± 40.16 (63.60–199.10)
Leaf R1		89.62 ± 32.92 (56.30–168.80)	90.78 ± 26.82 (56.00–139.80)
Root R1		22.56 ± 5.17 (14.90–30.53)	22.86 ± 7.17 (14.00–36.02)
Forage R4		37.95 ± 3.27 (33.20–42.20)	36.54 ± 2.70 (30.40–39.20)
Whole plant R6		12.88 ± 8.17 (4.02–33.60)	10.57 ± 5.63 (4.48–23.93)
Pollen R1		174.35 ± 21.64 (135.30–201.25)	174.66 ± 23.86 (145.20–230.15)
Grain R6		3.54 ± 1.26 (2.77–7.06)	3.51 ± 1.06 (2.61–6.27)
PAT			
Leaf V2–V4		18.23 ± 6.82 (7.61–26.66)	18.42 ± 7.64 (7.46–30.41)
Leaf V9		13.19 ± 6.46 (3.45–22.95)	15.11 ± 6.10 (7.65–27.31)
Leaf R1		13.30 ± 7.44 (4.23–29.33)	11.29 ± 3.15 (6.84–16.25)
Root R1		0.57 ± 0.26 (0.17–1.09)	0.58 ± 0.25 (0.22–1.15)
Forage R4		1.21 ± 0.58 (0.11–1.98)	1.10 ± 0.53 (0.09–1.81)
Whole plant R6		0.10 ± 0.06 ((0.05)–0.23)	0.06 ± 0.03 (ND–0.13)
Pollen R1		ND ± NA (ND–ND)	ND ± NA (ND–ND)
Grain R6		ND ± 0.03 (ND – 0.08)	(0.03) ± 0.03 (ND – 0.07)
AAD-1			
Leaf V2–V4	14.37 ± 6.11 (5.84–24.61)		14.20 ± 6.39 (6.01–23.73)
Leaf V9	6.60 ± 0.96 (4.73–8.01)		7.42 ± 3.04 (4.30–14.88)
Leaf R1	5.87 ± 2.22 (2.80–9.65)		7.47 ± 2.70 (4.66–11.67)
Root R1	2.69 ± 1.42 (1.03–4.19)		3.46 ± 2.12 (1.17–7.30)
Forage R4	6.84 ± 2.33 (3.18–10.29)		6.74 ± 2.46 (3.39–10.71)
Whole plant R6	3.31 ± 1.66 (0.89–5.73)		2.53 ± 1.16 (0.59–4.25)
Pollen R1	141.26 ± 21.81 (94.68–165.47)		139.21 ± 17.83 (103.64–159.20)
Grain R6	4.24 ± 1.43 (2.57–6.57)		3.87 ± 0.72 (2.64–4.95)