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Assessment of genetically modified maize MON 87427 × MON 89034 × MIR162 × MON 87411 and subcombinations, for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2017-144)

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Abstract

Maize MON 87427 × MON 89034 × MIR162 × MON 87411 (four-event stack maize) was produced by conventional crossing to combine four single events: MON 87427, MON 89034, MIR162 and MON 87411. The genetically modified organism (GMO) Panel previously assessed the four single maize events and four of the subcombinations and did not identify safety concerns. No new data on the single maize events or the four subcombinations that could lead to modification of 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 indicate that the combination of the single maize events and of the newly expressed proteins and dsRNA in the four-event stack maize does not give rise to food and feed safety and nutritional concerns. The GMO Panel concludes that the four-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 viable grains of the four-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 six maize subcombinations not previously assessed and concludes that these are expected to be as safe as and nutritionally equivalent to the single events, the previously assessed subcombinations and the four-event stack maize. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of the four-event stack maize. Post-market monitoring of food/feed is not considered necessary. The GMO Panel concludes that the four-event stack maize and its subcombinations are as safe as its non-GM comparator and 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-2017-144 under Regulation (EC) No 1829/2003 from Monsanto Company (referred to hereafter as the applicant), the Panel on Genetically Modified Organisms of the European Food Safety Authority (referred to hereafter as GMO Panel) was asked to deliver a Scientific Opinion on the safety of genetically modified glyphosate tolerant and insect resistant maize MON 87427 × MON 89034 × MIR162 × MON 87411 (referred to hereafter as 'four-event stack maize') and its subcombinations independently of their origin, according to Regulation (EU) No 503/2013 (referred to hereafter as 'subcombinations'). The scope of application EFSA–GMO–NL–2017–144 is for the placing on the market of maize MON 87427 × MON 89034 × MIR162 ×MON 87411 and all its subcombinations independently of their origin for food and feed uses, import and processing.

The term 'subcombination' refers to any combination of up to three of the events present in the four-event stack maize. The safety of subcombinations occurring as segregating progeny in the harvested grains of maize MON 87427 × MON 89034 × MIR162 × MON 87411 is evaluated in the context of the assessment of the four-event stack maize. The safety of subcombinations that have either been or could be produced by conventional crossing through targeted breeding approaches, and which can be bred, produced and marketed independently of the four-event stack, are risk assessed separately in the present scientific opinion.

The four-event stack maize was produced by conventional crossing to combine four single maize events: MON 87427 (expressing the 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein), MON 89034 (expressing the Cry1A.105 and Cry2Ab2 proteins), MIR162 (expressing the Vip3Aa20 and phosphomannose isomerase (PMI) proteins)) and MON 87411 (expressing the Cry3Bb1 and CP4EPSPS proteins, and the DvSnf7 dsRNA) to confer resistance to certain lepidopteran and coleopteran pests and tolerance to glyphosate-containing herbicides.

The GMO Panel evaluated the four-event stack maize and its subcombinations with reference to the scope and appropriate principles described in its guidelines for the risk assessment of GM plants and derived food and feed, the environmental risk assessment of GM plants and the post-market environmental monitoring (PMEM) of GM plants. The GMO Panel considered the information submitted in application EFSA-GMO-NL-2017-144, additional information provided by the applicant during the risk assessment, the scientific comments submitted by the Member States and the relevant scientific literature.

The previous assessments of the single events MON 87427, MON 89034, MIR162, MON 87411 and four of the subcombinations provided a basis for the assessment of the four-event stack maize and the remaining six subcombinations. No safety concerns were identified by the GMO Panel in the previous assessments. No safety issue concerning the four single maize events was identified by the updated bioinformatic analyses, nor reported by the applicant since the publication of the previous GMO Panel scientific opinions. Therefore, the GMO Panel considers that its previous conclusions on the safety of the single maize events remain valid.

For the four-event stack maize, the risk assessment included the molecular characterisation of the inserted DNA and analysis of protein expression. An evaluation of the comparative analysis 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 characterisation data establish that the events stacked in maize MON 87427 × MON 89034 × MIR162 × MON 87411 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins are similar in the four-event stack maize and in the single events, except for the expected difference for the CP4 EPSPS protein levels resulting from the combination of the MON 87427 and MON 87411 events, both producing CP4 EPSPS protein in the four-event stack. No indications of interactions that may affect the integrity of the events and the levels of the newly expressed proteins in this four-event stack maize were identified.

The comparative analysis of forage and grain composition and agronomic/phenotypic characteristics identified no differences between maize MON 87427 × MON 89034 × MIR162 × MON 87411 and the non-GM comparator that required further assessment for food/feed safety or environmental impact.

The molecular characterisation, the comparative analysis 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 and dsRNA in the four-event stack maize does not give rise to food and feed safety and nutritional concerns. The GMO Panel concludes that maize MON 87427 ×

MON 89034 × MIR162 × MON 87411, as described in this application, is as safe as and nutritionally equivalent to the non-GM comparator and the commercial non-GM maize reference varieties (hereafter 'non-GM reference varieties') tested.

Considering the combined events and their potential interactions, the outcome of the comparative analysis and the routes and levels of exposure, the GMO Panel concludes that maize MON 87427 × MON 89034 × MIR162 × MON 87411 would not raise safety concerns in the case of accidental release of viable GM maize grains into the environment.

Since no new safety concerns were identified for the four previously assessed subcombinations, and no new data leading to the modification of the original conclusions on safety were identified, the GMO Panel considers that its previous conclusions on these maize subcombinations remain valid. For the remaining six subcombinations included in the scope of application EFSA-GMO-NL-2017-144, no experimental data were provided. The GMO Panel assessed the possibility of interactions between the events in the six subcombinations and concludes that these subcombinations would not raise safety concerns. These subcombinations are therefore expected to be as safe as and nutritionally equivalent to the single events, the previously assessed subcombinations and the four-event stack maize.

Given the absence of safety concerns for foods and feeds from maize MON 87427 × MON 89034 × MIR162 × MON 87411 and its subcombinations, the GMO Panel considers that post-market monitoring of these products is not necessary. The PMEM plan and reporting intervals are in line with the intended uses of the four-event stack maize and its subcombinations. The literature searches did not identify any relevant publications on maize MON 87427 × MON 89034 × MIR162 × MON 87411. In the context of annual PMEM reports, the applicant could further improve future literature searches according to the GMO Panel recommendations provided in this scientific opinion.

The GMO Panel concludes that maize MON 87427 × MON 89034 × MIR162 × MON 87411 and its subcombinations, as described in this application, are as safe as the 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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1. Introduction

The scope of application EFSA-GMO-NL-2017-144 is for food and feed uses, import and processing in the European Union (EU) of the genetically modified (GM) herbicide-tolerant and insect-resistant maize MON 87427 × MON 89034 × MIR162 × MON 87411 and all its subcombinations independently of their origin.

1.1. Background

On 24 May 2017, the European Food Safety Authority (EFSA) received from the Competent Authority of The Netherlands application EFSA-GMO-NL-2017-144 for authorisation of maize MON 87427 × MON 89034 × MIR162 × MON 87411 (hereafter referred to as 'the four-event stack maize') (Unique Identifier MON–87427–79 × MON–89Ø34–39 × SYN-IR162-49 × MON-87411-9), submitted by Monsanto Europe S.A. (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003[1].

Following receipt of application EFSA-GMO-NL-2017-144, EFSA informed the Member States (MS) and the European Commission and made the summary of the application available to the public on the EFSA website.[2]

European Food Safety Authority (EFSA) checked the application for compliance with the relevant requirements of Regulation (EC) No 1829/2003 and Regulation (EU) No 503/2013[3] and, when needed, asked the applicant to supplement the initial application. On 13 July 2017, EFSA declared the application valid and made the application available to MS and the European Commission.

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 6 months to issue a scientific opinion on application EFSA-GMO-NL-2017-144. 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 MSs, including national Competent Authorities within the meaning of Directive 2001/18/EC.[4] The EU MSs had 3 months to make their opinion known on application EFSA-GMO-NL-2017-144 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 maize MON 87427 × MON 89034 × MIR162 × MON 87411 and all its subcombinations independently of their origin according to the of application EFSA-GMO-NL-2017-144.

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 including the opinions of the nominated risk assessment bodies of EU Member States.[5]

In addition to the present scientific opinion, EFSA and its GMO Panel were also asked to report on the particulars listed under Articles 6(5) and 18(5) of Regulation (EC) No 1829/2003. The relevant information is made available in the EFSA Register of Questions,[6] including the information required under Annex II to the Cartagena Protocol, a labelling proposal, a Post-Market Environmental

[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 at the EFSA Register of Questions: http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2017-00442

[3] Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorization of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. OJ L157, 8.6.2013, p. 1–48.

[4] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 12.3.2001, p. 1–38.

[5] Opinions of the nominated risk assessment bodies of EU Member States can be found at the EFSA Register of Questions (http://registerofquestions.efsa.europa.eu/roqFrontend/login), querying the assigned Question Number.

[6] http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2017-00442

Monitoring (PMEM) plan as provided by the applicant; the method(s), validated by the Community reference laboratory, for detection, including sampling, identification of the transformation event(s) in the food-feed and/or foods-feeds produced from it and the appropriate reference materials.

2. Data and methodologies

2.1. Data

The GMO Panel based its scientific assessment of maize MON 87427 × MON 89034 × MIR162 × MON 87411 on the valid application EFSA-GMO-NL-2017-144, additional information provided by the applicant during the risk assessment, relevant scientific comments submitted by EU MSs and relevant peer-reviewed scientific publications. As part of this comprehensive information package, the GMO Panel received additional unpublished studies submitted by the applicant in order to comply with the specific provisions of Regulation (EU) No 503/2013. A list of these additional unpublished studies is provided in Appendix B.

2.2. Methodologies

The GMO Panel conducted its assessment in line with the principles described in Regulation (EU) No 503/2013, its applicable guidelines (EFSA GMO Panel, 2010a,b 2011a,b, 2015a), explanatory notes and statements (EFSA, 2014, 2017a,b, 2019) for the risk assessment of GM plants. During its risk assessment, the GMO Panel considered all additional unpublished studies as listed in Appendix B for potential effects on human and animal health and the environment.

For the assessment of 90-day animal feeding studies, the GMO Panel took into account the criteria included in the EFSA guidance (EFSA Scientific Committee, 2011) and the explanatory statement for its applicability (EFSA, 2014).

The GMO Panel also assessed the applicant's literature searches, which include a scoping review, in accordance with the recommendations on literature searching outlined in EFSA (2010, 2017a, 2019).

In the frame of the contract OC/EFSA/GMO/2014/01, a contractor performed preparatory work and delivered report on the methods applied by the applicant in performing statistical analyses.

3. Assessment

3.1. Introduction

Application EFSA-GMO-NL-2017-144 covers the four-event stack maize MON 87427 × MON 89034 × MIR162 × MON 87411 and all its 10 subcombinations independently of their origin (Table 1).

Table 1: Stacked maize events covered by the scope of application EFSA-GMO-NL-2017-144

Degree of stacking	Events	Unique identifier
Four-event stack	MON 87427 × MON 89034 × MIR162 × MON 87411	MON–87427–79 × MON–89Ø34–39 × SYN-IR162-49 × MON-87411-9
Three-event stacks	MON 87427 × MON 89034 × MIR162	MON–87427–79 × MON–89Ø34–39 × SYN-IR162-49
	MON 87427 × MON 89034 × MON 87411	MON–87427–79 × MON–89Ø34–39 × MON-87411-9
	MON 87427 × MIR162 × MON 87411	MON–87427–79 × SYN-IR162-49 × MON-87411-9
	MON 89034 × MIR162 × MON 87411	MON–89Ø34–39 × SYN-IR162-49 × MON-87411-9
Two-event stacks	MON 87427 × MON 89034 ×	MON–87427–79 × MON–89Ø34–39
	MON 87427 × MIR162	MON–87427–79 × SYN-IR162-49
	MON 87427 × MON 87411	MON–87427–79 × MON-87411-9
	MON 89034 × MIR162	MON–89Ø34–39 × SYN-IR162-49
	MON 89034 × MON 87411	MON–89Ø34–39 × MON-87411-9
	MIR162 × MON 87411	SYN-IR162-49 × MON-87411-9

The term 'subcombination' refers to any combination of up to three of the maize events MON 87427, MON 89034, MIR162 and MON 87411.

The safety of subcombinations occurring as segregating progeny in harvested grains of maize MON 87427 × MON 89034 × MIR162 × MON 87411 is evaluated in the context of the assessment of the four-event stack maize in Section 3.5 of the present scientific opinion.

'Subcombination' also covers combinations 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 four-event stack maize. These subcombinations are assessed in Section 3.5 of this scientific opinion.

The four-event stack maize was produced by conventional crossing to combine four single maize events: MON 87427 (expressing the 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein), MON 89034 (expressing the Cry1A.105 and Cry2Ab2 proteins), MIR162 (expressing the Vip3Aa20 and PMI proteins) and MON 87411 (expressing the CP4 EPSPS and Cry3Bb1 proteins, and the DvSnf7 dsRNA) to confer resistance to certain lepidopteran and coleopteran pests and tolerance to glyphosate-containing herbicides. It should be noted that the assessment of herbicide residues in maize herbicide-tolerant crops relevant for this application has been investigated by the EFSA Pesticides Unit (EFSA, 2018).

All four single maize events, the two-event stacks MON 87427 × MON 89034, MON 87427 × MIR162, and MON 89034 × MIR162, and the three-event stack MON 87427 × MON 89034 × MIR162 have been previously assessed by the GMO Panel (see Table 2), and no safety concerns were identified.

Table 2: Single maize events and subcombinations of maize MON 87427 × MON 89034 × MIR162 × MON 87411 previously assessed by the GMO Panel

Event	Application or mandate	EFSA Scientific Opinion
MON89034	EFSA-GMO-NL-2007-37	EFSA GMO Panel (2008)
MIR162	EFSA-GMO-DE-2010-82	EFSA GMO Panel (2012)
MON 87427	EFSA-GMO-BE-2012-110	EFSA GMO Panel (2015b)
MON 87411	EFSA-GMO-NL-2015-124	EFSA GMO Panel (2018)
MON 89034 × MIR162	EFSA-GMO-NL-2016-131	EFSA GMO Panel (2019a,b)
MON 89034 × MON 87427	EFSA-GMO-BE-2013-117	EFSA GMO Panel (2017a)
MIR162× MON 87427	EFSA-GMO-NL-2016-131	EFSA GMO Panel (2019a,b)
MON 89034 × MIR162 × MON 87427	EFSA-GMO-NL-2016-131	EFSA GMO Panel (2019a,b)

3.2. Updated information on single events[7]

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

Updated bioinformatic analyses for maize events MON 87427, MON 89034, MIR162 and MON 87411 confirms that no known endogenous genes were disrupted by any of the inserts.

Updated bioinformatic analyses of the amino acid sequence of the newly expressed CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20, PMI and Cry3Bb1 proteins confirm previous results indicating no significant similarities to toxins and allergens. Updated bioinformatic analyses of the newly created Open Reading Frames (ORFs) within the inserts or spanning the junctions between the insert and the flanking regions for events MON 87427, MON 89034, MIR162 and MON 87411 confirmed previous analyses (Table 2). These analyses indicate that the production of a new peptide showing significant similarities to toxins or allergens for any of the events in maize MON 87427 × MON 89034 × MIR162 × MON 87411 is highly unlikely.

According to Regulation (EU) No 503/2013, when silencing approaches by RNAi have been used in GM plant applications, a bioinformatic analysis to identify potential 'off target' genes is required. The applicant has followed the recommendations by the GMO Panel for an RNAi off-target search in the four-stack maize expressing the DvSnf7 dsRNA.[8,9] Updated bioinformatics analysis confirms previous results that do not indicate an off-target effect of the DvSnf7 dsRNA expression that would need further assessment.

[7] Dossier. Part II – Sections 1.2.1.3 and 1.2.2.2; additional information on 6/12/2018 and 4/6/2019.
[8] Annex II of the minutes of the 118th GMO plenary meeting (https://www.efsa.europa.eu/sites/default/files/event/171025-m.pdf)
[9] Dossier: Part II – Section 1.2.1.3; additional information on 6/12/2018.

In order to assess the possibility for horizontal gene transfer (HGT) by homologous recombination (HR), the applicant performed a sequence identity analysis for events MON 87427, MON 89034, MIR162 and MON 87411 to microbial DNA. The likelihood and potential consequences of plant-to-bacteria gene transfer are described in Section 3.4.4.2.

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. Systematic literature review[10]

The GMO Panel assessed the applicant's literature searches on maize MON 87427 × MON 89034 × MIR162 × MON 87411, which included a scoping review, according to the guidelines given in EFSA (2010, 2017a, 2019).

A systematic review as referred to in Regulation (EU) No 503/2013 has not been provided in support to the risk assessment of application EFSA-GMO-NL-2017-144. Based on the outcome of the scoping review, the GMO Panel agrees that there is limited value in undertaking a systematic review for maize MON 87427 × MON 89034 × MIR162 × MON 87411 at present.

Although the overall quality of the performed literature searches is acceptable, the GMO Panel considers that the searches on maize MON 87427 × MON 89034 × MIR162 × MON 87411 could be improved. The GMO Panel therefore recommends the applicant to:

- ensure that enough search term variation is used (covering possible synonyms, related terms, acronyms, spelling variants, old and new terminology, brand and generic names, lay and scientific terminology, common typos, translation issues);
- ensure that enough truncation is used and used consistently.

The literature searches did not identify any relevant publications on maize MON 87427 × MON 89034 × MIR162 × MON 87411.

3.4. Risk assessment of the four-event stack maize MON 87427 × MON 89034 × MIR162 × MON 87411

3.4.1. Molecular characterisation

In line with the requirements laid down by Regulation (EU) No 503/2013, the possible impact of the combination of the events on the integrity of the events, the expression levels of the newly expressed proteins or the biological functions conferred by the individual inserts are considered below.

3.4.1.1. Genetic elements and their biological functions

Maize events MON 87427, MON 89034, MIR162 and MON 87411 were combined by conventional crossing to produce the four-event stack maize MON 87427 × MON 89034 × MIR162 × MON 87411. The structure of the inserts introduced into the four-event stack maize is described in detail in the respective EFSA scientific opinions (Table 2) and no new genetic modifications were involved. Genetic elements in the expression cassettes of the single events are summarised in Table 3.

Intended effects of the inserts in maize MON 87427 × MON 89034 × MIR162 × MON 87411 are summarised in Table 4.

Based on the known biological functions of the newly expressed proteins (Table 4), the only potential functional interactions at the biological level are among the Cry and Vip proteins.

[10] Dossier: Part II – Section 7; additional information: 3/10/2018, 5/12/2018, 21/6/2019.

Table 3: Genetic elements in the expression cassettes of the events stacked in maize MON 87427 × MON 89034 × MIR162 × MON 87411

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
MON 87427	35S (CaMV)	–	CTP2 (*Arabidopsis thaliana*)	*cp4 epsps (Agrobacterium sp.)*	*nos* (*Agrobacterium tumefaciens)*
MON 89034	35S (CaMV)	*cab* (*Triticum aestivum*)	–	*cry1A.105 (Bacillus thuringiensis)*	*hsp17* (*Triticum aestivum*)
	35S (FMV)	–	CTP (*Zea mays*)	*cry2Ab2 (Bacillus thuringiensis)*	*nos* (*Agrobacterium tumefaciens)*
MIR162	ZmUbiInt (*Zea mays*)	–	–	*vip3Aa20* (*Bacillus thuringiensis*)	35S (CaMV)
	ZmUbiInt (*Zea mays*)	–	–	*pmi* (*Escherichia coli*)	*nos* (*Agrobacterium tumefaciens)*
MON 87411	35S (CaMV)	–	–	*snf7* (*Diabrotica virgifera virgifera*)	E9 (*Pisum sativum*)
	pIIG (*Zea mays*)	*cab* (*Triticum aestivum*)	–	*cry3Bb1* (*Bacillus thuringiensis*)	*hsp17* (*Triticum aestivum*)
	TubA (*Oryza sativa*)	TubA (*Oryza sativa*)	CTP2 (*Arabidopsis thaliana*)	*cp4 epsps (Agrobacterium sp.*)	TubA (*Oryza sativa*)

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

Table 4: Characteristics and intended effects of the events stacked in maize MON 87427 × MON 89034 × MIR162 × MON 87411

Event	Protein/ dsRNA	Donor organism and biological function	Intended effects in GM plant
MON 87427	CP4 EPSPS	Based on a gene from *Agrobacterium* strain CP4 (Barry et al., 2001). 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 MON87427 expresses the bacterial CP4 EPSPS protein which confers tolerance to glyphosate-containing herbicides as it has lower affinity towards glyphosate than the plant endogenous enzyme
MON 89034	Cry1A.105	Based on a gene from *B. thuringiensis* subsp. *kurstaki* and subsp. *aizawai*. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes (Schnepf et al., 1998; Ellis et al., 2002)	Event MON 89034 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 *B. thuringiensis* subsp. *kurstaki*. *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 89034 expresses the Cry2Ab2, a protein toxic to certain lepidopteran larvae feeding on maize

Event	Protein/ dsRNA	Donor organism and biological function	Intended effects in GM plant
MIR162	Vip3Aa20	Based on a gene from *B. thuringiensis* strain AB88 (Estruch et al., 1996). In addition to Cry proteins, *B. thuringiensis* also produces insecticidal proteins during its vegetative growth stage. These are referred to as vegetative insecticidal proteins (Fang et al., 2007)	Event MIR162 expresses a modified version of the *B. thuringiensis vip3Aa1* gene, and encodes Vip3Aa20, a protein toxic to certain lepidopteran larvae feeding on maize
	PMI	Based on a gene from *E. coli strain* K-12. PMI (phosphomannose isomerase) catalyzes the isomerisation of mannose-6-phosphate to fructose-6-phosphate and plays a role in the metabolism of mannose (Markovitz et al., 1967)	Event MIR162 expresses PMI, which is used as selectable marker. Mannose normally inhibits root growth, respiration and germination. Transformed cells expressing PMI are able to utilise mannose as a carbon source (Negrotto et al., 2000)
MON 87411	DvSnf7 dsRNA	Based on a gene from western corn rootworm (WCR) (*Diabrotica virgifera virgifera* LeConte). The full-length Snf7 protein is part of the intracellular protein trafficking pathway (ESCRT) which is important for the maintenance of a functional intracellular transport of transmembrane proteins (Baum et al., 2007; Ramaseshadri et al., 2013)	Event MON87411 expresses DvSnf7 dsRNA which is a small RNA toxic to western corn rootworm feeding on maize
	Cry3Bb1	Based on a gene from *Bacillus thuringiensis*. *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 87411 expresses the Cry3Bb1, a protein toxic to certain coleopteran insects
	CP4 EPSPS	Based on a gene from *Agrobacterium* strain CP4 (Barry et al., 2001). 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 MON87411 expresses the bacterial CP4 EPSPS protein which confers tolerance to glyphosate-containing herbicides as it has lower affinity towards glyphosate than the plant endogenous enzyme

3.4.1.2. Integrity of the events in the four-event stack[11]

The genetic stability of the inserted DNA over multiple generations in the single maize events MON 87427, MON 89034, MIR162 and MON 87411 was demonstrated previously (see Table 2). Integrity of these events in maize MON 87427 × MON 89034 × MIR162 × MON 87411 was demonstrated by polymerase chain reaction (PCR) and sequence analysis that showed that the sequences of the events (inserts and their flanking regions) in the four-event maize stack are identical to the sequences originally reported for the four single events, thus confirming that the integrity of these events was maintained in the four-event stack maize.

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

CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20, PMI and Cry3Bb1 protein levels were analysed by enzyme-linked immunosorbent assay (ELISA) in material harvested from field trials at five locations in the USA in 2014. Samples analysed included leaves (V3-V4 and VT), roots (V3-V4 and R5), forage (R5), pollen (R1) and grain (R6) both those treated and not treated with glyphosate. In order to assess the changes in protein expression levels which may result from potential interactions between

[11] Dossier: Part II—Section 1.2.2.3 and additional information on 6/12/2018.
[12] Dossier: Part II—Section 1.2.2.3 and additional information on 6/12/2018, 9/4/2018, 4/6/2019.

the events, protein levels were determined for the four-event stack and the corresponding single events in different parts of the plant.

The levels of all the newly expressed proteins in the four-event stack maize and the corresponding singles were comparable in all tissues, except for CP4 EPSPS protein levels expected to be different because of the combination of events MON 87427 and MON 87411 both producing CP4 EPSPS in the four-event stack maize (Appendix A). Therefore, there is no indication of an interaction that may affect the levels of the newly expressed proteins in this stack.

The applicant provided a measure of the levels of DvSnf7 dsRNA in different tissues of the four-event maize stack and the single event MON 87411 including grain and forage. However, the dsRNA is an intermediate molecule which is processed by Dicer to siRNA molecules and the levels of dsRNA are not a good proxy for the levels of the active siRNAs in the plant (Pačes et al., 2017). Therefore, the levels of the DvSnf7 dsRNA were not considered relevant for the risk assessment of maize MON 87427 × MON 89034 × MIR162 × MON 87411.

3.4.1.4. Conclusions of the molecular characterisation

The molecular data establish that the events stacked in maize MON 87427 × MON 89034 × MIR162 × MON 87411 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins are comparable in the four-event stack and in the single events except for the expected higher levels of CP4 EPSPS protein in the four-event stack maize. Therefore, there is no indication of an interaction that may affect the integrity of the events or the levels of the newly expressed proteins in this stack. In addition, the potential impact of the DvSnf7 dsRNA on the levels of the newly expressed proteins was assessed by comparing the protein expression levels in the four-event stack and the respective singles. The data indicate that there is no impact of the DvSnf7 dsRNA on the expression levels of the newly expressed proteins.

Based on the known biological function of the newly expressed proteins, the only potential functional interactions are among the Cry and Vip proteins in susceptible insects, which will be addressed in Section 3.4.4.

3.4.2. Comparative analysis[13]

3.4.2.1. Overview of studies conducted for the comparative analysis

Application EFSA-GMO-NL-2017-144 presents data on agronomic and phenotypic characteristics, as well as on forage and grain composition of maize MON 87427 × MON 89034 × MIR162 × MON 87411 (Table 5).

Table 5: Overview of the comparative analysis studies to characterise the four-event stack maize in application EFSA-GMO-NL-2017-144

Study focus	Study details	Comparator	Non-GM reference varieties
Agronomic and phenotypic analysis	Field study, USA, 2014, 2016, 8 sites(a)	MPA640B	Twenty(c)
Compositional analysis	Field study, USA, 2014, 8 sites(b)		

(a): The field trials conducted in 2014 were located in Jefferson, IO; Stark, IL; Clinton, IL; Clinton, IN; Seward NE and Lehigh, PA. The field trials conducted in 2016 were located in Jefferson, IO and Perquimans, NC.

(b): The field trials were located in Jefferson, IO; Stark, IL; Clinton, IL; Vermilion, IL; Clinton, IN; Seward NE; Miami, OH and Lehigh, PA.

(c): Non-GM maize varieties used in the agronomic, phenotypic and compositional field trials, with their corresponding relative maturity indicated in brackets were Channel 211-97 (111), Dekalb DKC59-34 (109), Dekalb DKC62-06 (112), Gateway 6158 (115), LG2540 (108), LG2548 (108), LG2597 (112), Midland Phillips 7B15P (111), Mycogen 2H721 (112), Mycogen 2J790 (114), Mycogen 2M746 (113), NH6280 (112), NH6769 (115), Stewart S588 (112), Stewart S602 (112) and Stine 9724 (111). Channel 213-88 (113), Stewart S480 (107), Stine 9628 (109) and Specialty 3656 (111) were used only for the agronomic and phenotypic analysis where Dekalb DKC63-43 (113), NC+ 5220 (112) and Phillips 717 (109) for the compositional field trials only.

3.4.2.2. Experimental field trial design and statistical analysis

At each field trial site, the following materials were grown: the four-event maize, the comparator MPA640B and four non-GM hybrid maize reference varieties (hereafter 'non-GM reference varieties').

[13] Dossier: Part II – Section 1.3; additional information: 8/08/2018, 11/12/2018 and 9/04/2019.

All materials were treated with conventional herbicides management regimes; in addition, the field trials included the four-event stack maize exposed to the intended glyphosate-containing herbicide on top of the conventional herbicides.

The agronomic/phenotypic and compositional data were analysed as specified by EFSA GMO Panel (2010b, 2011a). This includes, for each of the two treatments of maize MON 87427 × MON 89034 × MIR162 × MON 87411, the application of a difference test (between the GM maize and the comparator) and an equivalence test (between the GM maize and the set of non-GM reference varieties).[14] The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).[15]

3.4.2.3. Suitability of selected test materials

Selection of the GM maize line and comparator

Event MON 87411 was originally produced in the inbred line LH244 (EFSA GMO Panel, 2018_AP124). The remaining three single events were transferred in the genetic background of two non-GM maize inbred lines: MIR162 was backcrossed into LH244; MON 87427 and MON 89039 into LH287.

In subsequent subsections, GM maize MON 87427 × MON 89034 × MIR162 × MON 87411 refers to hybrid obtained by crossing GM inbred line LH244 (carrying MIR162 and MON 87411) with GM inbred line LH287 (carrying MON 87427 and MON 89034).

The comparator used in the field trials is the non-GM maize hybrid (MPA640B [= LH244 × LH287]), which has a genetic background similar to that of maize MON 87427 × MON 89034 × MIR162 × MON 87411 (as documented by the pedigree), and is therefore considered to an appropriate comparator.

Both maize MON 87427 × MON 89034 × MIR162 × MON 87411 and the non-GM comparator MPA640B belong to a relative maturity 112, which is considered appropriate for growing in environments across North America, where the comparative field trials were conducted.

Selection of non-GM reference varieties

The 20 non-GM reference varieties with a relative maturity ranging from 107 to 115 were selected by the applicant and at each field trial site four of them were tested (see Table 5). On the basis of the information provided on relative maturity classes, the GMO Panel considers the selected non-GM reference varieties appropriate for the comparative assessment.

Seed production and quality

The seeds of the four-event stack maize and the comparator used in the field trials (see Table 5) were produced, harvested and stored under similar conditions, before being sown in the field trials. The seed lots of the four-event stack maize and the comparator were verified for their purity via event specific quantitative PCR analysis. The germination of four-event stack maize and the comparator was tested under warm and cold temperature conditions. The GMO Panel considers that the starting seed used as test material in the agronomic, phenotypic and compositional studies was of adequate quality.

Conclusion on suitability

The GMO Panel is of the opinion that the four-event stack maize, its comparator and the non-GM reference varieties were properly selected and of adequate quality. Therefore, the test materials are considered suitable for the comparative analysis.

3.4.2.4. Representativeness of the receiving environments

Selection of field trial sites

The selected field trial sites were located in commercial maize-growing regions of North America. The soil characteristics of the selected fields were diverse,[16] corresponding to optimal and near-optimal conditions for maize cultivation (Sys et al., 1993). The GMO Panel considers that the selected sites reflect commercial maize-growing regions in which the test materials are likely to be grown.

[14] The purpose of the test of equivalence is to evaluate the estimated mean values for the GM crop taking into account natural variability as defined by a set of non-GM references varieties with a history of safe use for consumption as food or feed.

[15] 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).

[16] Soil types of the field trials were clay loam, silty clay loam, loam and silt loam; soil organic matter ranged from 1.9% to 5.2%.

Meteorological conditions

Maximum and minimum mean temperatures and sum of precipitations were provided on a monthly basis. No exceptional weather conditions were reported at any of the selected field trial sites. The GMO Panel considers that the meteorological data set falls within the range of climatic conditions normally occurring at these sites.

Management practices

The field trials included plots containing the four-event stack maize, plots with the comparator and plots with non-GM reference varieties, all managed according to local agricultural practices. In addition, the field trials included plots containing four-event stack maize managed following the same agricultural practices, plus exposed to the intended glyphosate-containing herbicide that was applied at V3-V4 growth stage. The GMO Panel considers that the management practices including sowing, harvesting and application of plant protection products were appropriate.

Conclusion on representativeness

The GMO Panel concludes that the geographical locations, soil characteristics, meteorological conditions and management practices of the field trials are typical of the receiving environments where the test materials could be grown.

3.4.2.5. Agronomic and phenotypic analysis

Thirteen[17] agronomic and phenotypic endpoints plus information on abiotic stressors, disease incidence and arthropod damage were collected from the field trials (Table 5). The endpoints dropped ear count, stalk lodged plants and root lodged plants were not analysed as described in Section 3.4.2.2 because of insufficient variability in the data.

The outcome of the analysis for the remaining 10 endpoints was as follows:

- For the four-event stack maize (not treated with the intended herbicide), statistically significant differences with the non-GM comparator were identified for days to 50% pollen shed, days to 50% silking, ear height, grain moisture and yield. All these endpoints fell under equivalence category I or II.
- For the four-event stack maize (treated with the intended herbicide), statistically significant differences with the non-GM comparator were identified for early stand count, days to 50% pollen shed, days to 50% silking, ear height and grain moisture. All these endpoints fell under equivalence category I.

3.4.2.6. Compositional analysis

Maize forage and grains harvested from the field trial study in the USA in 2014 were analysed for 78 constituents (9 in forage and 69 in grain), including the key constituents recommended by OECD (OECD, 2002). The statistical analysis was not applied to 15 grain constituents[18] because more than half of the observations were below the limit of quantification.

The statistical analysis was applied to the remaining 63 constituents (9 in forage[19] and 54 in grain[20]); a summary of the outcome of the test of difference and the test of equivalence is presented in Table 6.

- For the four-event stack maize (not treated with the intended herbicide), significant differences with the non-GM comparator were identified for 47 endpoints (5 in forage and 42 in grain); of

[17] Early stand count, days to 50% pollen shed, days to 50% silking, stay green, ear height, plant height, dropped ear count, stalk lodged plants, root lodged plants, final stand count, grain moisture, test weight and yield.

[18] Caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), myristoleic acid (C14:1), pentadecanoic acid (C15:0), pentadecenoic acid (C15:1), heptadecanoic acid (C17:0), heptadecenoic acid (C17:1), gamma-linolenic acid (C18:3), eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3), arachidonic acid (C20:4), sodium and furfural.

[19] Ash, carbohydrates, protein, total fat, moisture, calcium, phosphorus, acid detergent fibre (ADF) and neutral detergent fibre (NDF).

[20] Proximates and fibre content (ash, carbohydrates, moisture, protein, total fat, acid detergent fibre (ADF), neutral detergent fibre (NDF) and total dietary fibre (TDF)), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium and zinc), vitamins (ß-carotene, thiamin, riboflavin, niacin, pyridoxine, folic acid and α-tocopherol), 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 (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C21:0) and behenic acid (C22:0)) and other compounds (phytic acid, raffinose, ferulic acid and *p*-coumaric acid).

those, ADF in forage fell under equivalence category III, while the other endpoints fell under category I/II.

- For the four-event stack maize (treated with the intended herbicide), significant differences with the non-GM comparator were identified for 35 endpoints (3 in forage and 32 in grain); all of them fell under category I/II.

Table 6: Outcome of the comparative compositional analysis of grains and forage from maize MON 87427 × MON 89034 × MIR162 × MON 87411. 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	15	46[d]	27	35[d]
	Category III/IV	–	1[e]	–	–
	Not categorised	1[f]	–	1[f]	–
	Total endpoints	63		63	

(a): Comparison between the four-event stack maize and the 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 glyphosate containing herbicide (see Section 3.4.2.4).
(d): Endpoints with significant differences between the four-event stack maize and its non-GM comparator and falling in equivalence category I–II. For grains, for both treated and non-treated GM: carbohydrates, protein, total fat, ADF, NDF, TDF, calcium, copper, magnesium, manganese, phosphorus, zinc, ß-carotene, niacin, pyridoxine, α-tocopherol, arginine, aspartic acid, glycine, phenylalanine, threonine, tyrosine, iron, palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), eicosenoic acid (C21:0), raffinose, ferulic acid and p-coumaric acid. Only non-treated: alanine, glutamic acid, histidine, isoleucine, leucine, lysine, proline, serine, tryptophan, valine and phytic acid. Only treated: linolenic acid (C18:3). For forage, for both treated and non-treated GM: carbohydrates, calcium and phosphorus. Non-treated only: NDF.
(e): Endpoints with significant differences between the four-event stack maize and its non-GM comparator and falling in equivalence category III/IV: ADF in forage (not treated only). Quantitative results are reported in Table 7.
(f): Endpoints not categorised for equivalence and without significant differences between the four-event stack maize and its non-GM comparator: total fat in forage (both treated and not treated).

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

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

Endpoint	Maize MON 87427 × MON 89034 × MIR162 × MON 87411		Comparator	Non-GM reference varieties	
	Not treated[a]	Treated[a]		Mean	Equivalence limits
ADF (% dw)	25.22*	23.86	23.42	22.94	20.87–25.01

dw: dry weight.
(a): Treated/not treated with the intended herbicide glyphosate.
For the four-event stack maize, significantly different values are marked with an asterisk, while the outcomes of the test of equivalence are differentiated by greyscale backgrounds: white (equivalence category I or II), light grey (equivalence category III) and dark grey (equivalence category IV).

3.4.2.7. Conclusions on the comparative analysis

Taking into account the natural variability observed for the set of non-GM reference varieties, the GMO Panel concludes that:

- None of the differences identified in agronomic and phenotypic characteristics tested between the four-event stack maize and the non-GM comparator needs further assessment regarding their potential environmental impact.
- None of the differences identified in forage and grain composition between the four-event stack maize and the non-GM comparator needs further food/feed safety assessment except for the change in levels of ADF in forage. This difference is further discussed in Section 3.4.3.

3.4.3. Food and Feed safety assessment[21]

3.4.3.1. Effect of processing

Maize MON 87427 × MON 89034 × MIR162 × MON 87411 will undergo existing production processes used for conventional maize. No novel production process is envisaged. Based on the outcome of the comparative assessment, processing of the four-event stack maize into food and feed products is not expected to result in products being different from those of conventional non-GM maize varieties.

3.4.3.2. Influence of temperature and pH on newly expressed proteins

Effects of temperature and pH on the newly expressed proteins in this four-event stack maize have been previously evaluated by the GMO Panel (Table 2).

3.4.3.3. Toxicology

Testing of newly expressed proteins

Six proteins (CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20, PMI and Cry3Bb1) are newly expressed in maize MON 87427 × MON 89034 × MIR162 × MON 87411 (Section 3.4.1). The GMO Panel has previously assessed these proteins in the context of the single events (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 this conclusion.

The potential for a functional interaction between the proteins newly expressed in maize MON 87427 × MON 89034 × MIR162 × MON 87411 has been assessed with regard to human and animal health. The insecticidal proteins Cry1A.105, Cry2Ab2 and Cry3Bb1 are delta-endotoxins acting through cellular receptors found in target insect species. It is reported that the gastrointestinal tract of mammals, including humans, lacks receptors with high specific affinity to Cry proteins (Hammond et al., 2013; Koch et al., 2015). The Vip3Aa20 protein is a protein secreted by *B. thuringiensis* during its vegetative phase acting in target insects via a mechanism similar to that of Cry proteins (Chakroun et al., 2016; Bel et al., 2017). The CP4 EPSPS and PMI proteins are enzymes that catalyse distinct biochemical reactions and act on unrelated substrates in the plant with high substrate specificity.

On the basis of the known biological function of the individual newly expressed proteins (Table 4), there is currently no expectation for possible interactions relevant to the food and feed safety of maize MON 87427 × MON 89034 × MIR162 × MON 87411.

In vitro protein degradation studies on CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20, PMI and Cry3Bb1 proteins have been previously evaluated by the GMO Panel (Table 2).

The GMO Panel concludes that there are no safety concerns to human and animal health related to the newly expressed proteins CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20, PMI and Cry3Bb1 in maize MON 87427 × MON 89034 × MIR162 × MON 87411.

Testing of new constituents other than newly expressed proteins

No new constituents other than newly expressed proteins have been identified in maize MON 87427 × MON 89034 × MIR162 × MON 87411, with the exception of the intended expression of DvSnf7 dsRNA and derived siRNAs, designed to control coleopteran pests via RNAi. According to the applicant, the gene-silencing effects of DvSnf7 dsRNA are mainly driven by ingestion of dsRNA from the plant and its processing into siRNAs by the insects. The GMO Panel has previously assessed DvSnf7 dsRNA and derived siRNAs in the context of the single event (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 this conclusion.

[21] Additional information 4/6/2019 and additional information 16/10/2018, 13/11/2018, 4/06/2019, 21/06/2019, 20/08/2019.

Information on altered levels of food and feed constituents

Acid detergent fibre (ADF) levels in forage were significantly different in maize MON 87427 × MON 89034 × MIR162 × MON 87411 not treated with the conventional herbicide when compared with its non-GM comparator and showed a lack of equivalence with the non-GM reference varieties (Section 3.4.2.6). No toxicological concern is identified regarding this compositional change. Further information on safety is provided in Section 3.4.3.6.

Testing of the whole genetically modified food and feed

Based on the outcome of the molecular characterisation, comparative analysis and toxicological assessment, no indication of findings relevant to food/feed safety related to the stability and expression of the inserts or to interaction between the transformation events, and no modifications of toxicological concern in the composition of maize MON 87427 × MON 89034 × MIR162 × MON 87411 have been identified (see Sections 3.4.1.4, 3.4.2.7 and 3.4.3. Therefore, animal studies on food/feed derived from maize MON 87427 × MON 89034 × MIR162 × MON 87411 are not necessary (EFSA GMO Panel, 2011a).

In accordance to Regulation (EU) No 503/2013, the applicant provided a 90-day oral repeated-dose toxicity study in rats on whole food and feed from each of the maize single-event MON 87427, MON 89034, MIR162 and MON 87411. The four studies had already been provided in the context of the single-event applications and assessed by the GMO Panel (Table 2); no adverse effects related to the administration of the respective GM diets had been identified. In the context of the assessment of maize MON 87427 × MON 89034 × MIR162 × MON 87411 and in order to fulfil the requirements of Regulation (EU) No 503/2013 for 90-day studies, upon EFSA's request, the applicant provided additional information for maize single events MON 87427, MON 89034 and MIR162.

The GMO Panel has previously assessed the above-mentioned additional information on MON 87427, MON 89034 and MIR162 in the context of another applications under Regulation (EU) 503/2013 (EFSA GMO Panel, 2019a,b_AP131). The conclusion was that these studies are in line with the requirements of Regulation (EU) 503/2013 and that there are no indications of adverse effects related to the 90-day administration to rats of diets including up to 41.5% grains from maize MON87427, MON89034 and MIR162.

3.4.3.4. 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; Regulation (EU) 503/2013). 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. Furthermore, an assessment of specific newly expressed proteins in relation to their potential to cause celiac disease was also performed (EFSA GMO Panel, 2017c).

Assessment of allergenicity of the newly expressed proteins

For allergenicity, the GMO Panel has previously evaluated the safety of CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20, PMI and Cry3Bb1 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 four-event stack maize affecting their allergenicity are expected.

For adjuvanticity, the Bt protein Cry1Ac has been suggested to possess adjuvant activity based on animal studies when applied at relatively high doses (e.g. Vázquez et al., 1999; Santos-Vigil et al., 2018). The Panel has previously evaluated the safety of Cry1A.105, Cry2Ab2, Vip3Aa20 and Cry3Bb1 proteins, and no concerns on adjuvanticity were identified in the context of the applications assessed (see Table 2). More recently, this aspect has been discussed in detail by EFSA (EFSA, 2018; Parenti et al., 2019). The levels of the individual Bt proteins in the four-event stack maize are comparable to those in the respective single maize events (see Section 3.4.1.4). From the limited evidence available,

the GMO Panel does not find indications that the presence of the Bt proteins at the levels expressed in this four-event stack maize might act as adjuvants with the potential to enhance a specific immunoglobulin E (IgE) response and to favour the development of an allergic reaction.

The applicant provided spontaneous information on the safety of the CP4 EPSPS, Cry2Ab2, Cry1A.105 and Cry3Bb1 proteins regarding their potential hazard to cause a celiac disease response.[21,22] For such assessment, the applicant followed the principles described in the EFSA GMO Panel guidance document (2017c). The assessment of the CP4 EPSPS and Cry2Ab2 proteins identified no perfect or relevant partial matches with known celiac disease peptide sequences. The assessment of the Cry1A.105 revealed partial matches which have been previously evaluated by the GMO Panel (EFSA GMO Panel, 2019a_AP134). The assessment of the Cry3Bb1 proteins revealed partial matches containing the Q/E-X1-P-X2 motif and requiring further investigations. Based on additional considerations on position and nature of amino acids flanking the QLPV motif, such as the absence of prolines at specific positions and the charge and size of adjacent amino acids (EFSA GMO Panel, 2017c), the two relevant peptides containing the motif do not raise concern as they fail to mimic gluten sequences. Therefore, no indications of safety concerns were identified by the GMO Panel.

Assessment of allergenicity of GM plant products

The GMO Panel regularly reviews the available publications on food allergy to maize. However, maize is not considered a common allergenic food[23] (OECD, 2002). Therefore, the GMO Panel does 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 3.4.1, 3.4.2 and 3.4.3), the GMO Panel identifies no indications of a potentially increased allergenicity of food and feed derived from this four-event stack maize with respect to that derived from the non-GM comparator.

3.4.3.5. Dietary exposure assessment to new constituents

In line with Regulation (EU) No 503/2013, the applicant provided dietary exposure estimates to CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20, PMI and Cry3Bb1 proteins newly expressed in MON 87427 × MON 89034 × MIR162 × MON 87411 maize. Dietary exposure was estimated based on protein expression levels reported in this application for the four-event stack maize treated with the intended herbicide, the current available consumption data and feed practices, the foods and feeds currently available in the market and the described processing conditions.

Dietary exposure to DvSnf7 dsRNA was not estimated, in line with the approach followed for the single event (Table 2).

Table 8 describes the protein expression levels used to estimate both human and animal dietary exposure.

[22] It is pointed out that the requirements laid down in the recent EFSA guidance on allergenicity (2017) are not applicable to this dossier, as described in Section 1.5 'Transition period' of the guidance document.

[23] 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.

Table 8: Mean values (n = 20, μg/g dry weight and μg/g fresh weight) for newly expressed proteins in grains and forage and pollen from MON 87427 × MON 89034 × MIR162 × MON 87411 maize treated with the intended herbicide[(a)]

Protein	Tissue/developmental stage		
	Grains/R6 (μg/g dry weight and μg/g fresh weight)	Pollen/R1 (μg/g fresh weight)[(b)]	Forage/R5 (μg/g dry weight)
CP4 EPSPS	13[(c)]/11	17	230
Cry1A.105	2.2[(c)]/2.0	5.4	15
Cry2Ab2	2.3[(c)]/2.0	0.4	47
Cry3Bb1	6.7[(c)]/5.9	31	62
PMI	1.3/1.1[(d)]	1.6[(d)]	5.2
Vip3Aa20	52/46[(d)]	[(d)]	97

(a): Intended herbicide: glyphosate.
(b): Concentration values in pollen were adjusted to 6% moisture content before using them to estimate dietary exposure to the different newly expressed protein via the consumption of pollen supplements (reported moisture content for pollen = 35%).
(c): Concentration values estimated originally in fresh weight were converted into μg/g dry weight using a standard dry weight conversion factor (DWCF) of 0.88 to account for approximately 12% moisture content in the grains.
(d): Fresh weight values for Vip3Aa20 and PMI proteins used to estimate human dietary exposure were calculated by multiplying the dry weight values by a DWCF of 0.88 to account for approximately 12% moisture content in the grains and by a factor of 0.65 to account for approximately 35% moisture content in pollen.

Human dietary exposure[24]

Human dietary exposure was estimated across different European countries on different population groups: young population (infants, toddlers, 'other children'), adolescents, adult population (adults, elderly and very elderly) and special populations (pregnant and lactating women).

For the purpose of estimating dietary exposure, the levels of newly expressed proteins in MON 87427 × MON 89034 × MIR162 × MON 87411 maize grains were derived from replicated field trials (four replicates from five locations) in the 2014 US growing season. Mean values (fresh weight basis) are considered as the most to estimate dietary exposure (see Table 8). Since no specific consumption data were available on commodities containing, consisting of or obtained from MON 87427 × MON 89034 × MIR162 × MON 87411 maize grains, a conservative scenario with 100% replacement of conventional maize by the GM maize was considered. Consumption figures for all relevant commodities (e.g. corn flakes, sweet corn, popcorn, etc.) were retrieved from the EFSA Comprehensive European Food Consumption Database (EFSA consumption database).[25] Maize oil was excluded from the assessment since no proteins are expected to be present in the oil.

For the acute dietary exposure estimations, the applicant directly assigned to processed commodities the mean value reported for the concentration of the newly expressed proteins in maize grains. This is a conservative approach as neither recipes nor the effect of processing on the final concentration of newly expressed proteins are considered. Summary statistics from the EFSA consumption database were used.[26] Acute dietary exposure in high consumers within each dietary survey and age class was estimated by summing the exposure derived from the 95th percentile consumption for the dominant food commodity[27] among consumers only and those exposures derived from the mean consumption of the remaining food categories in the total population (EFSA, 2015). Table 9 shows the highest acute dietary exposure for the different newly expressed proteins; highest dietary exposure estimates ranged between 3.9 μg/kg body weight (bw) per day for PMI in adults (18–65 years) and 373 μg/kg bw per day for Vip3Aa20 in toddlers (1–3 years). The most relevant food commodities in terms of contribution to the exposure were sweet corn (toddlers) and popcorn (adults).

[24] Dossier: Part II – Section 2.4 and additional information 21/06/2019.
[25] http://www.efsa.europa.eu/en/data/food-consumption-data
[26] Summary statistics from the EFSA Comprehensive European Food Consumption Database accessed in September 2016.
[27] Dominant food commodity refers to the food that will lead to the highest exposure among all consumed foods.

Table 9: Highest acute dietary exposure to CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20, PMI and Cry3Bb1 proteins (μg/kg bw per day) estimated across European dietary surveys and different age classes

	Acute dietary exposure (μg/kg bw per day)					
	CP4 EPSPS	**Cry1A.105**	**Cry2Ab2**	**Cry3Bb1**	**Vip3Aa20**	**PMI**
Toddlers	89	16.2	16.2	47.8	373	8.9
Adults	39	7.0	7.0	20.7	161	3.9

bw: body weight.

The GMO Panel estimated chronic dietary exposure to CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20, PMI and Cry3Bb1 proteins. Individual consumption data of the relevant food commodities were retrieved from the EFSA Consumption Database, using dietary surveys with at least 2 days consumption and covering a total of 22 European countries.[28] Different recipes and factors were considered to estimate the amount of maize in the consumed commodities before assigning CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20, PMI and Cry3Bb1 proteins levels to the relevant commodities.[29] No losses in the NEPs during processing were considered, except for certain commodities excluded from the exposure estimations (maize oil, corn starch, corn syrup). The 95th percentile chronic exposure (highly exposed population) was derived from the distribution of the individual dietary exposure estimates within each dietary survey and age class.

Table 10 shows the chronic dietary exposure to each of the newly expressed proteins across European dietary surveys; dietary exposure ranged between 0.03 μg/kg bw per day for PMI protein in elderly population (65–74 years old) and 206.8 μg/kg bw per day for Vip3Aa20 protein in infants (< 1 year). Main average contributors to the exposure in the dietary surveys with the highest estimates were sweet corn in infants, and cornflakes in toddlers and 'other children'.

Table 10: Range of chronic dietary exposure estimates (95th percentiles, highly exposed population) to CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20, PMI and Cry3Bb1 proteins (μg/kg bw per day) across European dietary surveys and different age classes

		Chronic dietary exposure (μg/kg bw per day)					
	N	**CP4 EPSPS**	**Cry1A.105**	**Cry2Ab2**	**Cry3Bb1**	**Vip3Aa20**	**PMI**
Infants	11	0.0–49.5	0.0–9.0	0.0–9.0	0.0–26.5	0.0–206.8	0.0–4.9
Toddlers	14	2.7–46.0	0.5–8.4	0.5–8.4	1.4–24.7	11.3–192.3	0.3–4.6
Other children	19	7.3–40.3	1.3–7.3	1.3–7.3	3.9–21.6	30.5–168.6	0.7–4.0
Adolescents	18	1.6–30.2	0.3–5.5	0.3–5.5	0.8–16.2	6.6–126.2	0.2–3.0
Adults	19	0.7–15.2	0.1–2.8	0.1–2.8	0.4–8.1	3.1–63.5	0.1–1.5
Elderly	18	0.3–9.3	0.1–1.7	0.1–1.7	0.1–5.0	1.2–39.1	0.03–0.9
Very elderly	14	0.0–8.7	0.0–1.6	0.0–1.6	0.0–4.7	0.0–36.3	0.0–0.9
Pregnant women	2	6.1–22.0	1.1–4.0	1.1–4.0	3.3–11.8	25.6–92.2	0.6–2.2
Lactating women	2	4.5–17.7	0.8–3.2	0.8–3.2	2.4–9.5	18.7–74.1	0.4–1.8

bw: body weight; N: number of dietary surveys.

An *ad hoc* dietary exposure scenario was carried out for consumers of pollen supplements under the assumption that these supplements are made of pollen from maize MON 87427 × MON 89034 × MIR162 × MON 87411 (Table 11). Consumption data on pollen supplements were available for few consumers across five different European countries;[25] the low number of consumers available adds uncertainty to the exposure estimations. Among consumers of pollen supplements, the average acute dietary exposure would range from 0.003 μg/kg bw per day for Cry2Ab2 and 43.2 μg/kg bw per day for Vip3Aa20, in the elderly population in both cases. Similarly, the average chronic dietary exposure would range from 0.001 μg/kg bw per day for Cry2Ab2 and 28.8 μg/kg bw per day for Vip3Aa20, also

[28] Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Germany, Denmark, Estonia, Finland, France, United Kingdom, Greece, Croatia, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Spain, Romania, and Sweden.

[29] 100 grams of maize bread are made with approximately 74 g of maize flour, and a reverse yield factor of 1.22 from the conversion of maize grains into flour is used. This results in 41.5 μg of Vip3Aa20 per gram of maize bread as compared to 46 μg/g in the maize grains.

in the elderly population. Dietary exposure in high consumers of pollen supplements (based on 95th percentile consumption) was not estimated as the limited consumption data available prevents from deriving robust statistical estimates.

Table 11: Dietary exposure to CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20, PMI and Cry3Bb1 proteins (μg/kg bw per day) in consumers of pollen across different age classes

	Average exposure (μg/kg bw per day)					
	CP4 EPSPS	**Cry1A.105**	**Cry2Ab2**	**Cry3Bb1**	**Vip3Aa20**	**PMI**
Chronic dietary exposure	0.1–12.1	0.02–3.9	0.001–0.3	0.1–22.8	0.1–28.8	0.01–1.2
Acute dietary exposure	0.1–18.1	0.04–5.9	0.003–0.4	0.2–34.2	0.3–43.2	0.01–1.8

bw: body weight.

Animal dietary exposure[24]

Animal dietary exposure to CP4EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20, PMI and Cry3Bb1 proteins was estimated following the consumption of maize grain, gluten feed, gluten meal and maize forage/silage since these are the maize products entering the feed chain. A conservative scenario with 100% replacement of conventional maize products by the GM products was considered.

Mean levels of CP4EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20, PMI and Cry3Bb1 proteins in maize grains and forage/silage were derived from replicated field trial sites (five locations) in the 2014 US growing season (Table 8). To estimate the mean NEP levels in maize gluten feed and gluten meal, a factor of 2.6 and 7.1 folds, respectively, was applied, based on the protein content of gluten feed and gluten meal relative to maize grain (OECD, 2002), assuming that no losses of NEP occur during processing.

Dietary exposure to CP4EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20, PMI and Cry3Bb1 proteins in maize MON 87427 × MON 89034 × MIR162 × MON87411 following the consumption of maize grain, gluten feed and gluten meal was provided by the applicant across different animal species (i.e. broiler, finishing swine and dairy cattle), based on estimates for animal body weight, daily feed intake and inclusion rates (percentage) of maize grain, gluten feed and gluten meal in animal diets (OECD, 2009). Estimated dietary exposure was calculated for each feed material and reported as the sum of their consumption through diets, as detailed in Table 12.

Table 12: Dietary exposure to CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20, PMI and Cry3Bb1 proteins (μg/kg bw per day) in livestock

	Dietary exposure (μg/kg bw per day)					
	CP4 EPSPS	**Cry1A.105**	**Cry2Ab2**	**Vip3Aa20**	**PMI**	**Cry3Bb1**
Broiler	**1,532**	**259**	**271**	**6,130**	**153**	**790**
Finishing swine	**753**	**127**	**133**	**3,011**	**75**	**388**
Dairy cattle	**1,250**	**212**	**221**	**5,000**	**125**	**644**

The GMO Panel estimated dietary exposure to CP4EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20, PMI and Cry3Bb1 proteins across different livestock animal species (beef and dairy cattle, lamb, breeding swine and layer) following the consumption of maize forage/silage, based on estimates for animal body weight, daily feed intake and inclusion rates of maize forage/silage in animal diets (OECD, 2009). Estimated dietary exposure in livestock is reported in Table 13.

Table 13: Dietary exposure to CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20, PMI and Cry3Bb1 proteins (μg/kg bw per day) in livestock

	Dietary exposure (μg/kg bw per day)					
	CP4 EPSPS	**Cry1A.105**	**Cry2Ab2**	**Vip3Aa20**	**PMI**	**Cry3Bb1**
Beef cattle	4,416	288	902	1,862	100	1,190
Dairy cattle	5,307	346	1,085	2,238	120	1,430
Lamb	2,932	191	599	1,237	66	790
Breeding swine	1,061	69	217	448	24	286
Layer	1,574	103	321	664	35	424

3.4.3.6. Nutritional assessment of endogenous constituents

The intended traits of maize MON 87427 × MON 89034 × MIR162 × MON 87411 are herbicide tolerance and insect resistance, with no intention to alter nutritional parameters. However, levels of ADF in forage (not treated) were significantly different from the non-GM comparator and showed a lack of equivalence with the set of non-GM reference varieties (Section 3.4.2.6).

Animal nutrition

The increase in ADF percentage reported in Table 7 for the GM forage does not represent a safety issue for animals. Herbivorous animals consume feed materials with high amount of fibre; therefore, the minimal difference observed is not considered relevant. From these data, the GMO Panel concludes that the nutritional impact of maize MON 87427 × MON 89034 × MIR162 × MON 87411-derived food and feed is similar to that expected from the non-GM comparator and non-GM reference varieties.

3.4.3.7. Conclusion on the food and feed safety assessment

The newly expressed proteins Cry1A.105, Cry2Ab, Cry3bB1, PMI, Vip3Aa20, CP4 EPSPS and the DvSnf7 dsRNA and derived siRNAs in the four-event stack maize MON 87427 × MON 89034 × MIR162 × MON 87411 do not raise safety concerns for human and animal health. No interactions between the newly expressed proteins relevant for food and feed safety were identified. Similarly, the GMO Panel did not identify indications of safety concerns regarding allergenicity or adjuvanticity related to the presence of the newly expressed proteins in maize MON 87427 × MON 89034 × MIR162 × MON 87411, or regarding the overall allergenicity of this four-event stack maize. Based on the outcome of the comparative assessment and the nutritional assessment, the GMO Panel concludes that the nutritional impact of maize MON 87427 × MON 89034 × MIR162 × MON 87411-derived food and feed is expected to be the same as those derived from the non-GM comparator and non-GM reference varieties. The GMO Panel concludes that four-event stack maize MON 87427 × MON 89034 × MIR162 × MON 87411 as described in this application, is nutritionally equivalent to and as safe as the non-GM comparator and the non-GM reference varieties tested.

3.4.4. Environmental risk assessment[30]

Considering the scope of application EFSA-GMO-NL-2017-144, which excludes cultivation, the environmental risk assessment (ERA) of maize MON 87427 × MON 89034 × MIR162 × MON 87411 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 maize MON 87427 × MON 89034 × MIR162 × MON 87411 grains during transportation and/or processing (EFSA GMO Panel, 2010b).

3.4.4.1. 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, 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 maize MON 87427 × MON 89034 × MIR162 × MON 87411 will provide a selective advantage to maize plants, except when they are exposed to glyphosate-containing herbicides or infested by insect pests that are susceptible to the DvSnf7 dsRNA or to the Cry1A.105, Cry2Ab2, Vip3Aa20 and/or Cry3Bb1 proteins. However, this fitness advantage will not allow the GM plant to overcome other biological and abiotic factors (described above) limiting plant's persistence and invasiveness. Therefore, the presence of the intended traits will not affect the persistence and invasiveness of the GM plant.

[30] Dossier: Part II – Section 5; additional information 4/6/2019.

In conclusion, the GMO Panel considers it very unlikely that maize MON 87427 × MON 89034 × MIR162 × MON 87411 will differ from conventional maize hybrid varieties in its ability to survive until subsequent seasons, or to establish occasional feral plants under European environmental conditions in case of accidental release into the environment of viable maize MON 87427 × MON 89034 × MIR162 × MON 87411 grains.

3.4.4.2. Potential for gene transfer

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer (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). This assessment included consideration of homology-based recombination processes, as well as non-homologous end joining and microhomology-mediated end joining. Possible fitness advantages that the bacteria in the receiving environments would gain from acquiring recombinant DNA were considered. No concern was identified in regard to 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.

The applicant submitted an updated bioinformatic analysis for each of the single events to assess the possibility for HGT by homologous recombination.

The updated bioinformatics analyses of events MON 87427, MON 89034, MIR162 and MON 87411 do not reveal any new DNA sequence that could provide sufficient length and identity which could facilitate HGT by double homologous recombination, confirming the conclusions of previous Scientific Opinions (EFSA GMO Panel, 2018_AP124, 2019a_AP134).

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 four-event stack maize to bacteria does not raise any environmental safety concern.

Plant-to-plant gene transfer

The potential for occasional feral GM maize MON 87427 × MON 89034 × MIR162 × MON 87411 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 3.4.4.1). 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.

3.4.4.3. Interactions of the GM plant with target organisms

Taking the scope of application EFSA-GMO-NL-2017-144 (no cultivation), potential interactions of occasional feral four-event stack maize plants arising from grain import spills with the target organisms are not considered a relevant issue.

3.4.4.4. 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 four-event stack maize grains is limited, and because ingested dsRNA and proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions of the four-event stack maize with non-target organisms are not considered by the GMO Panel to raise any environmental safety concern. Interactions that may occur among the Cry and Vip proteins (as mentioned in Section 3.4.1.4) will not alter this conclusion.

3.4.4.5. Interactions with abiotic environment and biogeochemical cycles

Given that environmental exposure to spilled grains or occasional feral four-event stack maize plants arising from grain import spills is limited, and because ingested dsRNA and 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 by the GMO Panel to raise any environmental safety concern.

3.4.4.6. Conclusion of the environmental risk assessment

The GMO Panel concludes that it is unlikely that the four-event stack maize would differ from conventional maize varieties in its ability to persist under European environmental conditions. Considering the scope of the application EFSA-GMO-NL-2017-144, interactions of occasional feral four-event stack maize plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from the four-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 four-event stack maize would not raise safety concerns in the event of accidental release of viable GM maize grains into the environment.

3.4.5. Conclusion on the four-event stack maize MON 87427 × MON 89034 × MIR162 × MON 87411

No new data on the four single maize events MON 87427, MON 89034, MIR162 and MON 87411 that would lead to a modification of the original conclusions on their safety were identified.

The combination of maize events MON 87427, MON 89034, MIR162 and MON 87411 in the four-event stack maize did not give rise to issues concerning the molecular, agronomic/phenotypic or compositional characteristics of the four-event stack maize that would be of concern for food and feed safety and nutrition.

The newly expressed proteins and the DvSnf7 dsRNA in the four-event stack maize do not raise safety concerns for human and animal health and the environment in light of the scope of this application.

No indications of interactions between the events based on the biological functions of the newly expressed proteins that would raise a safety issue were identified in maize MON 87427 × MON 89034 × MIR162 × MON 87411. Comparison of the levels of the newly expressed proteins between the four-event stack maize and those of the single maize events did not reveal an interaction at protein expression level. In addition, the potential impact of the DvSnf7 dsRNA on the levels of the newly expressed proteins was assessed by comparing the protein expression levels in the four-event stack and the respective singles. The data indicate that there is no impact of the DvSnf7 dsRNA on the expression levels of the newly expressed proteins.

Considering the combined traits and their potential interactions, the outcome of the comparative analysis and the routes and levels of exposure, the GMO Panel concludes that maize MON 87427 × MON 89034 × MIR162 × MON 87411 would not raise safety concerns in the event of accidental release of viable GM maize grains into the environment.

No scientific information that could change the conclusions on this four-event stack maize was retrieved through systematic literature searches covering the 10 years before submission of the application and the period since the time of validity of the application. The GMO Panel concludes that maize MON 87427 × MON 89034 × MIR162 × MON 87411, as described in this application, is nutritionally equivalent to and as safe as the comparator and the non-GM reference varieties tested.

3.5. Risk assessment of the subcombinations

Subcombinations previously assessed in the frame of other applications are discussed in Section 3.5.1. The strategy followed for the subcombinations that have not been previously assessed (Section 3.5.2) has been described by the GMO Panel.[31] In this case, the risk assessment takes as its starting point the assessment of the single maize events, and uses the data generated for the four-event stack as well as all the additional data available on subcombinations previously assessed by the GMO Panel (Table 2).

3.5.1. Subcombinations previously assessed

The GMO Panel has previously assessed four subcombinations and no safety concerns were identified the two-event maize stacks MON89034 × MON87427, MON 89034 × MIR162; and MON87427 × MIR162, and the three-event stack maize MON 87427 × MON 89034 × MIR162 (see Table 2). Literature searches covering the 10 years before submission of the application (January 2006–June 2018) and the period since the time of validity of the application revealed no new scientific information relevant to the risk assessment of these maize stacks.[32] Consequently, the GMO Panel considers that its previous conclusions on these subcombinations remain valid.

3.5.2. Subcombinations not previously assessed

Six of the 10 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 14).

Table 14: Maize stacks not previously assessed and covered by the scope of application EFSA GMO NL-2017-144

Degree of stacking	Events
Three-event stack	MIR162 × MON 87427 × MON 87411
	MON 89034 × MON 87427 × MON 87411
	MON 89034 × MIR162 × MON 87411
Two-event stack	MON 87427 × MON 87411
	MIR162 × MON 87411
	MON 89034 × MON 87411

3.5.2.1. Stability of the events

The genetic stability of the inserted DNA over multiple generations in the four single maize events was demonstrated previously (see Table 2). Integrity of the events was demonstrated in the four-event stack maize MON 87427 × MON 89034 × MIR162 × MON 87411 (Section 3.4.1.2) and the previously assessed maize subcombinations (EFSA GMO Panel, 2017a,b, 2019a,b). The GMO Panel finds no reasons to expect the loss of integrity of the events in the maize subcombinations not previously assessed (see Table 14).

3.5.2.2. Expression of the events

The GMO Panel assessed whether any combination of the four 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 six subcombinations compared with those in the single maize events. This assumption was confirmed by comparing the levels of the newly expressed proteins of each single maize event with those of the four-event stack maize. The levels were similar in the four-event stack maize and in the single events except for CP4 EPSPS, which showed, in general, the expected higher level in the stack resulting from the combination of the single events MON 87427 and MON 87411 (Section 3.4.1.3 and Appendix A). In addition, the potential impact of the DvSnf7 dsRNA on the levels

[31] 115th GMO Panel meeting (Annex 1 of the minutes: http://www.efsa.europa.eu/sites/default/files/event/170517-m.pdf).
[32] Dossier: Part II – Section 7; additional information: 8/08/2018.

of the newly expressed proteins was assessed by comparing the protein expression levels in the four-event stack and the respective singles. The data indicate that there is no impact of the DvSnf7 dsRNA on the expression levels of the newly expressed proteins. This supports the conclusion that interactions affecting the expression levels of the newly expressed proteins are not expected in the six subcombinations not previously assessed and included in the scope of application EFSA-GMO-NL-2017-144.

3.5.2.3. Potential functional interactions between the events

The GMO Panel assessed the potential for interactions between maize events in the six subcombinations not previously assessed (Table 14), taking into consideration intended traits and unintended effects.

Based on the known biological functions of the individual newly expressed proteins and dsRNA (Table 4), there is currently no expectation for possible interactions relevant for the food and feed or environmental safety between these proteins in those subcombinations. The GMO Panel took into account all the intended and potential unintended effects considered in the assessment of the four single events, the previously assessed subcombinations (Table 2) and the four-event stack maize. It is concluded that none of these events would raise safety concerns when combined in any of these maize subcombinations. The GMO Panel considers that no further data are needed to complete the assessment of subcombinations from the four-event stack maize.

3.5.3. Conclusion

Since no new safety concerns were identified for the previously assessed subcombinations, the GMO Panel considers that its previous conclusions on these maize subcombinations remain valid. For the remaining six subcombinations included in the scope of application EFSA-GMO-NL-2017-144, no experimental data have been provided. For these subcombinations, the GMO Panel assessed the possibility of interactions between the events and concluded that these combinations would not raise safety concerns. These subcombinations are therefore expected to be as safe as and nutritionally equivalent to the single maize events, the previously assessed subcombinations and the four-event stack maize.

3.6. Post-market monitoring

3.6.1. Post-market monitoring of GM food/feed

The GMO Panel concluded that the four-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 (Section 3.4.3.7). Four of the subcombinations have been previously assessed and no safety concerns were identified. The six subcombinations not previously assessed and included in the scope of application EFSA GMO NL 2017 144 are expected to be as safe as and nutritionally equivalent to the single maize events, the previously assessed maize subcombinations and the four-event stack maize (Section 3.5.3). Therefore, the GMO Panel considers that post-market monitoring of food and feed from the four-event stack maize and its subcombinations, as described in this application, is not necessary.

3.6.2. Post-market environmental monitoring[33]

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

Monitoring is related to risk management, and thus, a final adoption of the PMEM plan falls outside the mandate of EFSA. However, the GMO Panel gives its opinion on the scientific 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 four-event stack mazie, no case-specific monitoring is required.

[33] Dossier: Part II – Section 6.

The PMEM plan proposed by the applicant for the four-event stack maize includes: (1) the description of a monitoring approach involving operators (federations involved in import and processing), reporting to the applicant, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment; (2) a coordinating system established by EuropaBio for the collection of information recorded by the various operators; and (3) the review of relevant scientific publications retrieved from literature searches (Lecoq et al., 2007; Windels et al., 2008). The applicant proposes to submit a PMEM report on an annual basis and a final report at the end of the authorisation period.

The GMO Panel considers that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of the four-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 four-event stack maize and its subcombinations.

In the context of annual PMEM reports, the applicant should improve future literature searches according to the GMO Panel recommendations given in Section 3.3.

3.6.3. Conclusions on post-market monitoring

No post-market monitoring of 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 maize MON 87427 × MON 89034 × MIR162 × MON 87411.

4. Overall conclusions

The GMO Panel was asked to carry out a scientific assessment of maize MON 87427 × MON 89034 × MIR162 × MON 87411 and subcombinations for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003.

No new information on the four single maize events MON 87427, MON 89034, MIR162 and MON 87411 that would lead to a modification of the original conclusions on their safety were identified.

The molecular characterisation, the 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 and the dsRNA in the four-event stack maize does not give rise to food/feed safety and nutritional concerns. The GMO Panel concludes that the four-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.

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

Since no new data on the four subcombinations previously assessed that would lead to a modification of the original conclusions on their safety were identified, the GMO Panel considers that its previous conclusions on these maize stacks remain valid. For the remaining six subcombinations included in the scope of application EFSA-GMO-NL-2017-144, no information has been provided. The GMO Panel assessed possible interactions between the events in the six subcombinations, and concludes that these combinations of events MON 87427, MON 89034, MIR162, and MON 87411 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 four-event stack maize.

The literature searches did not identify any relevant publications on maize MON 87427 × MON 89034 × MIR162 × MON 87411. In the context of annual PMEM reports, the applicant could further improve future literature searches according to the GMO Panel recommendations.

In addition, the GMO Panel considered the additional unpublished studies listed in Appendix B. This new information does not raise any concern for human and animal health and the environment regarding the four-event stack maize and its subcombinations.

Given the absence of safety and nutritional concerns for foods and feeds from the four-event stack maize and all its subcombinations, the GMO Panel considers that PMM of these products is not necessary. The PMEM plan and reporting intervals are in line with the intended uses of the four-event stack maize and its subcombinations.

In conclusion, the GMO Panel considers that maize MON 87427 × MON 89034 × MIR162 × MON 87411 and its subcombinations, as described in this application, are as safe as the non-GM

comparator and the tested non-GM reference varieties with respect to potential effects on human and animal health and the environment.

Documentation as provided to EFSA

- Letter from the Competent Authority of Netherlands received on 24 May 2017 concerning a request for authorisation of the placing on the market of maize MON 87427 × MON 89034 × MIR162 × MON 87411 (EFSA-GMO-NL-2017-144) submitted in accordance with Regulation (EC) No 1829/2003 by Monsanto Europe S.A./N.V.
- Application EFSA-GMO-NL-2017-144 validated by EFSA, 13 July 2017.
- Application stopped due to single event, 13 July 2017.
- Application restarted following adoption of single event, 31 May 2018.
- Request for supplementary information to the applicant, 07 June 2018.
- Request for supplementary information to the applicant, 07 June 2018.
- Request for supplementary information to the applicant, 02 August 2018.
- Receipt of supplementary information from the applicant, 08 August 2018.
- Receipt of supplementary information from the applicant, 24 September 2018.
- Receipt of supplementary information from the applicant, 03 October 2018.
- Request for supplementary information to the applicant, 15 October 2018.
- Receipt of supplementary information from the applicant, 16 October 2018.
- Request for supplementary information to the applicant, 08 November 2018.
- Receipt of supplementary information from the applicant, 13 November 2018.
- Request for supplementary information to the applicant, 15 November 2018.
- Receipt of supplementary information from the applicant, 05 December 2018.
- Receipt of supplementary information from the applicant, 06 December 2018.
- Receipt of supplementary information from the applicant, 11 December 2018.
- Request for supplementary information to the applicant, 05 February 2019.
- Receipt of supplementary information from the applicant, 09 April 2019.
- Request for supplementary information to the applicant, 18 April 2019.
- Request for supplementary information to the applicant, 22 May 2019.
- Receipt of supplementary information from the applicant, 04 June 2019.
- Receipt of supplementary information from the applicant, 21 June 2019.
- Receipt of spontaneous information from the applicant, 22 August 2019.

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Abbreviations

ADF	acid detergent fibre
CaMV	cauliflower mosaic virus
CTP	Chloroplast transit peptide
ELISA	enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	environmental risk assessment
FMV	Figwort Mosaic Virus
GM	genetically modified
GMO	genetically modified organism
GMO Panel	EFSA Panel on Genetically Modified Organisms
HGT	horizontal gene transfer
HR	homologous recombination
IgE	immunoglobulin E
NDF	neutral detergent fibre
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PCR	polymerase chain reaction
PMEM	post-market environmental monitoring
PMI	phosphomannose isomerase
TDF	total dietary fibre
UTR	untranslated region

Appendix A – Protein expression data

Means, standard deviation and ranges of protein levels (μg/g dry weight) from maize MON 87427 × MON 89034 × MIR162 × MON 87411 (treated with glyphosate), MON 87427 (treated with glyphosate), MON 89034 (not treated), MIR162 (not treated) and MON 87411 (treated with glyphosate), from field trials performed across five locations in USA in 2014[(a)]

Protein	Event(s)	Leaf (V3-V4)	Leaf (VT)	Root (V3-V4)	Forage Root (R5)	Forage (R5)	Pollen (R1)	Grain (R6)
CP4 EPSPS	**MON 87427 × MON 89034 × MIR162 × MON 87411**	940[(b)] ± 310[(c)] 150–1,500[(d)]	1,000 ± 140 730–1,300	260 ± 56 170–360	190 ± 41 110–280	230 ± 63 140–340	26 ± 4.3 20–33	13 ± 1.7 8.2–16
	MON87427	870 ± 130 700–1,300	870 ± 86 680–1,100	200 ± 53 120–300	160 ± 40 76–230	180 ± 44 96–270	N/A	8.6 ± 1.3 6.1–11
	MON87411	56 ± 7.3 43–69	45 ± 2.8 41–53	51 ± 16 27–81	21 ± 9.3 5.7–38	12 ± 3.4 6.7–19	23 ± 3.0 17–28	4.4 ± 0.49 3.5–5.3
Cry1 A.105	**MON 87427 × MON 89034 × MIR162 × MON 87411**	200 ± 46 130–290	88 ± 37 50–180	48 ± 15 29–85	10 ± 2.2 7.0–15	15 ± 4.6 5.7–27	8.4 ± 1.1 6.6–10	2.2 ± 0.23 1.8–2.7
	MON89034	180 ± 33 140–240	73 ± 18 49–120	39 ± 11 24–58	10 ± 2.2 5.6–14	13 ± 4.7 3.0–20	8.4 ± 0.96 6.7–10	2.1 ± 0.32 1.6–2.8
Cry2Ab2	**MON 87427 × MON 89034 × MIR162 × MON 87411**	200 ± 23 150–240	150 ± 28 110–200	52 ± 19 21–83	27 ± 7.0 12–37	47 ± 13 20–77	0.57 ± 0.083 0.45–0.75	2.3 ± 0.37 1.5–3.0
	MON89034	190 ± 28 140–240	120 ± 24 88–160	41 ± 13 21–62	26 ± 7.5 11–36	35 ± 9.5 22–54	0.61 ± 0.12 0.45–0.84	2.2 ± 0.44 1.4–3.1
Vi3Aa20	**MON 87427 × MON 89034 × MIR162 × MON 87411**	170 ± 41 30–220	110 ± 14 83–130	110 ± 38 60–200	36 ± 9.2 24–54	97 ± 33 49–180	62 ± 6.5 52–75	52 ± 8.5 36–68
	MIR162	220 ± 31 170–310	110 ± 26 45–140	95 ± 25 65–150	35 ± 8.2 20–52	98 ± 20 73–150	64 ± 4.9 57–75	54 ± 10 38–71
PMI	**MON 87427 × MON 89034 × MIR162 × MON 87411**	8.8 ± 2.7 1.5–14	9.4 ± 2.1 6.6–15	6.3 ± 3.1 2.8–14	2.9 ± 0.99 1.6–5.1	5.2 ± 1.7 2.7–9.5	2.5 ± 0.26 2.0–3.1	1.3 ± 0.23 0.91–1.8
	MIR162	9.7 ± 1.5 7.4–13	9.4 ± 2.2 5.5–13	7.2 ± 1.4 5.1–11	2.7 ± 0.61 1.8–4.2	5.3 ± 1.1 3.8–7.4	2.4 ± 0.24 1.9–2.8	1.3 ± 0.17 0.90–1.6
Cry3Bb1	**MON 87427 × MON 89034 × MIR162 × MON 87411**	360 ± 41 300–450	200 ± 39 130–270	280 ± 84 120–410	75 ± 36 29–150	62 ± 18 40–110	49 ± 7.0 37–61	6.7 ± 1.0 4.0–8.9
	MON87411	310 ± 48 200–410	180 ± 52 45–250	260 ± 80 130–440	72 ± 29 26–130	56 ± 17 24–85	50 ± 8.1 36–69	7.2 ± 4.7 4.2–27

(a): Number of samples is n = 20, except for: n = 1 for pollen (for CP4 EPSPS in MON 87427), n = 19 for OSL1 (for CP4EPSPS and Cry3Bb1 in MON 87411) and n = 19 for OSL4 (for Vip3Aa20 and PMI in MIR162).
(b): Mean.
(c): Standard deviation.
(d): Range.

Appendix B – Additional studies

List of additional studies performed by or on behalf of the applicant with regard to the evaluation of the safety of maize MON 87427 × MON 89034 × MIR162 × MON 87411 for humans, animal or the environment

Study identification	Title
MSL0022958	The Effect of Heat Treatment on Cry2Ab2 Immunodetection
SSB-507-07 A1	Evaluation of MIR162 Transgenic Maize Grain in a Broiler Chicken Feeding Study
MSL0027023	Amended Report for MSL0026697: Assessment of DvSnf7 RNA Levels in Maize Tissues Collected from MON 87427 × MON 89034 × MIR162 × MON 87411 Produced in the United States Field Trials During 2014
MSL0026942	Assessment of DvSnf7 RNA Levels in Maize OSL1, OSL4, OSR1, Forage Root, Forage, Pollen, and Grain Tissues Collected from MON 87427 × MON 89034 × MIR162 × MON 87411 Produced in United States Field Trials During 2014
MSL0028504	Amended Report for MSL0028257: An Evaluation of the Potential for Interaction between the Lepidopteran Insecticidal Traits in the Combined Maize Product MON 87427 × MON 89034 × MIR162 × MON 87411 with Corn Earworm (*Helicoverpa zea*)
MSL0026614	Compositional Analyses of Maize Forage and Grain from MON 87427 × MON 89034 × MIR162 × MON 87411 Grown in the United States in 2014
MSL0023074	The Effect of Heat Treatment on Cry3Bb1 Immunodetection
MSL0027526	Comparison of Lipid Transfer Protein (LTP) Expression Levels from MON 87427 × MON 89034 × MIR162 × MON 87411 with Conventional Control Maize
MSL0022940	Immunodetection of Cry1A.105 Following Heat Treatment
MSL0028515	Amended Report for MSL0028185: Assessment of CP4 EPSPS, Cry1A.105, Cry2Ab2, Cry3Bb1, Vip3Aa20 and PMI Protein Levels in Maize Tissues Collected from MON 87427 × MON 89034 × MIR162 × MON 87411, MON 87427, MON 89034, MIR162 and MON 87411 Produced in Brazilian Field Trials During Safrinha 2016
MSL00026328	Comparison of Broiler Performance and Carcass Parameters When Fed Diets Containing MON 87427 × MON 89034 × MIR162 × MON 87411 (Stack), Control, or Reference Maize Grain
MSL0028034	Amended Report for MSL0027183: An Evaluation for the Potential for Interaction between the Lepidopteran and Coleopteran Traits Produced by the Combined Maize Product MON 87427 × MON 89034 × MIR162 × MON 87411 with the Corn Earworm (Helicoverpa zea)
MSL0027182	An Evaluation of the Protein for Interaction among Plant Incorporated Protectants Produced by MON 87427 × MON 89034 × MIR162 × MON 87411 with the Southern Corn Rootworm, (Diabrotica undecimpunctata howardi)
MSL0028199	Assessment of DvSnf7 RNA Levels in Maize Tissues Collected from MON 87427 × MON 89034 × MIR162 × MON 87411 and MON 87411 Produced in Brazilian Field Trials during 2016
MSL0026733	Southern Blot Analyses to Confirm the Presence of MIR162 in the Combined Trait Maize Product MON 87427 × MON 89034 × MIR162 × MON 87411
MSL0027308	Southern Blot Analyses to Confirm the Presence of MON 87427, MON 89034 and MON 87411 in the Combined Trait Maize Product MON 87427 × MON 89034 × MIR162 × MON 87411