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Assessment of genetically modified maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 and subcombinations, for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2016-134)

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Abstract

Maize MON 87427 ×MON 87460 × MON 89034 × MIR162 × NK603 (five-event stack maize) was produced by conventional crossing to combine five single events: MON 87427, MON 87460, MON 89034, MIR162 and NK603. The GMO Panel previously assessed the five single maize events and eleven of the subcombinations and did not identify safety concerns. No new data on the single maize events or the 11 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 in the five-event stack maize does not give rise to food and feed safety and nutritional concerns. The GMO Panel concludes that the five-event stack maize, as described in this application, is as safe as and nutritionally equivalent to its non-GM comparator and the non-GM reference varieties tested. In the case of accidental release of viable grains of the five-event stack maize into the environment, this would not raise environmental safety concerns. The GMO Panel assessed the likelihood of interactions among the single events in the 14 maize subcombinations 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 five-event stack maize. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of the five-event stack maize. Post-market monitoring of food/feed is not considered necessary. The GMO Panel concludes that the five-event stack maize and its subcombinations are as safe as its non-GM comparator and the tested non-GM reference varieties with respect to potential effects on human and animal health and the environment.

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Summary

Following the submission of application EFSA-GMO-NL-2016-134 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 drought- and glyphosate-tolerant and insect resistant maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 (referred to hereafter as 'five-event stack maize') and its subcombinations independently of their origin, according to the Commission Regulation (EU) No 503/2013 (referred to hereafter as 'subcombinations'). The scope of application EFSA–GMO–NL–2016–134 is for the placing on the market of maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 and all its subcombinations independently of their origin for food and feed uses, import and processing.

The term 'subcombination' refers to any combination of up to four of the events present in the five-event stack maize. The safety of subcombinations occurring as segregating progeny in the harvested grains of maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 is evaluated in the context of the assessment of the five-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 five-event stack, are risk assessed separately in the present scientific opinion.

The five-event stack maize was produced by conventional crossing to combine five single maize events: MON 87427 (expressing the 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein); MON 87460 (expressing the cold shock protein B (CSPB) and neomycin phosphotransferase II protein (NPTII)); MON 89034 (expressing the Cry1A.105 and Cry2Ab2 proteins); MIR162 (expressing the Vip3Aa20 and phosphomannose isomerase (PMI) proteins)); and NK603 (expressing the CP4 EPSPS protein and the variant CP4 EPSPS L214P) to confer resistance to certain lepidopteran pests and tolerance to drought and glyphosate-containing herbicides.

The GMO Panel evaluated the five-event stack maize and its subcombinations with reference to the scope and appropriate principles described in its guidelines for the risk assessment of GM plants and derived food and feed, the environmental risk assessment of GM plants and the post-market environmental monitoring of GM plants. The GMO Panel considered the information submitted in application EFSA-GMO-NL-2016-134, 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 87460, MON 89034, MIR162, NK603 and eleven of the subcombinations provided a basis for the assessment of the five-event stack maize and the remaining 14 subcombinations. No safety concerns were identified by the GMO Panel in the previous assessments. No safety issue concerning the five single maize events was identified by the updated bioinformatic analyses, nor reported by the applicant since the publication of the previous GMO Panel scientific opinions. Therefore, the GMO Panel considers that its previous conclusions on the safety of the single maize events remain valid.

For the five-event stack maize, the risk assessment included the molecular characterisation of the inserted DNA and analysis of protein expression. An evaluation of the comparative 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 post-market environmental monitoring (PMEM) plan was also undertaken.

The molecular data establish that the events stacked in maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins are similar in the five-event stack maize and in the single events, except for the expected difference for the CP4 EPSPS protein levels resulting from the combination of the MON 87427 and NK603 single events, both producing CP4 EPSPS protein in the five-event stack. No indications of interactions that may affect the integrity of the events and the levels of the newly expressed proteins in this five-event stack maize were identified.

The comparative analysis of forage and grain composition and agronomic/phenotypic characteristics identified no differences between maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 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 in the five-event stack maize does not give rise to food and feed safety and nutritional concerns. The GMO Panel concludes that maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603, as described in this application, is as safe as and nutritionally equivalent to its non-GM comparator and the non-GM reference varieties tested.

Considering the combined 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 87460 × MON 89034 × MIR162 × NK603 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 eleven 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 14 subcombinations included in the scope of application EFSA-GMO-NL-2016-134, no experimental data were provided. The GMO Panel assessed the possibility of interactions between the events in the 14 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 five-event stack maize.

Based on the relevant publications identified through the literature searches, the GMO Panel does not identify any safety issue pertaining to the intended uses of maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 and its subcombinations. In the context of annual PMEM reports, the applicant could further fine–tune future literature searches according to the GMO Panel recommendations given in this scientific opinion.

Given the absence of safety concerns for foods and feeds from maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 and its subcombinations, the GMO Panel considers that post-market monitoring of these products is not necessary. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of the five-event stack maize and its subcombinations.

The GMO Panel concludes that maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 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-2016-134 is for food and feed uses, import and processing in the European Union (EU) of the genetically modified (GM) herbicide- and drought-tolerant and insect-resistant maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 and all its subcombinations independently of their origin.

1.1. Background

On 30 November 2016, the European Food Safety Authority (EFSA) received from the Competent Authority of The Netherlands application EFSA-GMO-NL-2016-134 for authorisation of maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 (hereafter referred to as 'the five-event stack maize') (Unique Identifier MON-87427-7 × MON-8746Ø-4 × MON-89Ø34-3 × SYN-IR162-4 × MON-ØØ6Ø3-6), 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-2016-134, 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]

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 19 January 2017, EFSA declared the application valid and made the valid application available to MS and the EC.

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-2016-134. Such time limit was extended whenever EFSA and/or its GMO Panel requested supplementary information to the applicant. According to Regulation (EC) No 1829/2003, any supplementary information provided by the applicant during the risk assessment was made available to the EU Member States and European Commission (for further details, see the section 'Documentation', below).

In accordance with Regulation (EC) No 1829/2003, EFSA consulted the nominated risk assessment bodies of EU Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC.[4] The EU Member States had three months to make their opinion known on application EFSA-GMO-NL-2016-134 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 87460 × MON 89034 × MIR162 × NK603 and all maize subcombinations of the individual events independently of their origin (as present in the segregating progeny as well as independent stacks to be placed on the market as such) in the context of its scope as defined in application EFSA-GMO-NL-2016-134.

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 on maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603, 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 Monitoring (PMEM) plan as

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.
[2] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2016-00686
[3] Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. OJ L157, 8.6.2013, p. 1–48.
[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-2016-00686

provided by the applicant; the method(s), validated by the Community reference laboratory, for detection, including sampling, identification of the transformation event 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 risk assessment of maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 on the valid application EFSA-GMO-NL-2016-134, additional information provided by the applicant during the risk assessment, relevant scientific comments submitted by EU Member States and relevant peer-reviewed scientific publications. In addition to this comprehensive information package, the GMO Panel also received 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 (i.e., EFSA GMO Panel, 2010a,b, 2011a,b, 2015a) and explanatory notes (i.e. EFSA, 2017a,b) 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 of the GM food and feed 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 2011 EFSA Scientific Committee guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed (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).

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

3. Assessment

3.1. Introduction

Application EFSA-GMO-NL-2016-134 covers the five-event stack maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 and all its 25 subcombinations independently of their origin (Table 1). The scope of this application is for food and feed uses, import and processing, and excludes cultivation within the European Union (EU).

Table 1: Stacked maize events covered by the scope of application EFSA-GMO-NL-2016-134

Degree of stacking	Event	Unique identifier
Five-event stack maize	MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603	MON–87427–7 × MON 8746Ø × MON–89Ø34–3 × SYN-IR162-4 × MON–ØØ6Ø3–6
Four-event stack maize	MON 87460 × MON 89034 × MIR162 × NK603	MON 8746Ø × MON–89Ø34–3 × SYN-IR162-4 × MON–ØØ6Ø3–6
	MON 87427 × MON 89034 × MIR162 × NK603	MON–87427–7 × MON–89Ø34–3 × SYN-IR162-4 × MON–ØØ6Ø3–6
	MON 87427 × MON 87460 × MIR162 × NK603	MON–87427–7 × MON 8746Ø × SYN-IR162-4 × MON–ØØ6Ø3–6
	MON 87427 × MON 87460 × MON 89034 × NK603	MON–87427–7 × MON 8746Ø × MON–89Ø34–3 × MON–ØØ6Ø3–6
	MON 87427 × MON 87460 × MON 89034 × MIR162	MON–87427–7 × MON 8746Ø × MON–89Ø34–3 × SYN-IR162-4

Degree of stacking	Event	Unique identifier
Three-event stack maize	MON 87427 × MON 87460 × MON 89034	MON–87427–7 × MON 8746Ø × MON–89Ø34–3
	MON 87427 × MON 87460 × MIR162	MON–87427–7 × MON 8746Ø × SYN-IR162-4
	MON 87427 × MON 87460 × NK603	MON–87427–7 × MON 8746Ø × MON–ØØ6Ø3–6
	MON 87427 × MON 89034 × NK603	MON–87427–7 × MON–89Ø34–3 × MON–ØØ6Ø3–6
	MON 87427 × MIR162 × NK603	MON–87427–7 × SYN-IR162-4 × MON–ØØ6Ø3–6
	MON 87427 × MON 89034 × MIR162	MON–87427–7 × MON–89Ø34–3 × SYN-IR162-4
	MON 87460 × MON 89034 × MIR162	MON–87427–7 × MON–89Ø34–3 × SYN-IR162-4
	MON 87460 × MON 89034 × NK603	MON 8746Ø × MON–89Ø34–3 × MON–ØØ6Ø3–6
	MON 87460 × MIR162 × NK603	MON 8746Ø × SYN-IR162-4 × MON–ØØ6Ø3–6
	MON 89034 × MIR162 × NK603	MON–89Ø34–3 × SYN-IR162-4 × MON–ØØ6Ø3–6
Two-event stack maize	MON 87427 × MON 89034	MON–87427–7 × MON–89Ø34–3
	MON 87427 × MON 87460	MON–87427–7 × MON 8746Ø
	MON 87427 × NK603	MON–87427–7 × MON–ØØ6Ø3–6
	MON 87427 × MIR162	MON–87427–7 × SYN-IR162-4
	MON 87460 × MON 89034	MON 8746Ø × MON–89Ø34–3
	MON 87460 × MIR162	MON 8746Ø × SYN-IR162-4
	MON 87460 × NK 603	MON 8746Ø × MON–ØØ6Ø3–6
	MON 89034 × NK603	MON–89Ø34–3 × MON–ØØ6Ø3–6
	MON 89034 × MIR162	MON–89Ø34–3 × SYN-IR162-4
	MIR162 × NK603	SYN-IR162-4 × MON–ØØ6Ø3–6

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

The safety of subcombinations occurring as segregating progeny in the harvested grains of maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 is evaluated in the context of the assessment of the five-event stack maize in Section 3.4 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 five-event stack maize. These subcombinations are risk assessed in the Section 3.5 of this scientific opinion.

The five-event stack maize was produced by conventional crossing to combine five single maize events: MON 87427 (expressing the 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein); MON 87460 (expressing the cold shock protein B (CSPB) and Neomycin phosphotransferase II protein (NPTII)); MON 89034 (expressing the Cry1A.105 and Cry2Ab2 proteins); MIR162 (expressing the Vip3Aa20 and phosphomannose isomerase (PMI) proteins)); and NK603 (expressing the CP4 EPSPS protein and the variant CP4 EPSPS L214P) to confer resistance to certain lepidopteran pests and tolerance to drought and glyphosate-containing herbicides. It should be noted that the assessment of herbicide residues relevant for this application has been investigated by the EFSA Pesticides Unit (EFSA, 2018).

All five single maize events, the two-event stack MON 89034 × NK603, the three-event stack maize MON 87427 × MON 89034 × NK603 and all its subcombinations as well as the four-event stack maize MON 87427 × MON 89034 × MIR162 × NK603 and all its subcombinations independently of their origin have been previously assessed by the GMO Panel (see Table 2), and no safety concerns were identified.

Table 2: Single maize events and subcombinations of maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 previously assessed by the GMO Panel

Event	Application or mandate	EFSA Scientific Opinion
MON 87427	EFSA-GMO-BE-2012-110	EFSA GMO Panel (2015b)
MON 87460	EFSA-GMO-NL-2009-70	EFSA GMO Panel (2012a)
MON 89034	EFSA-GMO-NL-2007-37	EFSA (2008)
MIR162	EFSA-GMO-DE-2010-82	EFSA GMO Panel (2012b)
NK603	CE/ES/00/01 EFSA-GMO-NL-2005-22 EFSA-GMO-RX-NK603	EFSA (2004, 2007) EFSA (2009) EFSA (2009)
MON 89034 × NK603	EFSA-GMO-NL-2007-38	EFSA GMO Panel (2009)
MON 87427 × MON 89034 × NK603 and subcombinations	EFSA-GMO-BE-2013-117	EFSA GMO Panel, (2017a)
MON 87427 × MON 89034 × MIR162 × NK603 and subcombinations	EFSA-GMO-NL-2016-131	EFSA GMO Panel (2019)

3.2. Updated information on the single events[7]

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

Updated bioinformatic analyses for maize events MON 87427, MON 87460, MON 89034, MIR162 and NK603, confirm that no known endogenous genes were disrupted by any of the inserts.

Updated bioinformatic analyses of the amino acid sequence of the newly expressed CP4 EPSPS, CSPB, NPTII, Cry1A.105, Cry2Ab2, Vip3Aa20 and PMI proteins confirmed previous results indicating no significant similarities to toxins or allergens. Updated bioinformatics analyses of the newly created open reading frames (ORFs) within the inserts or spanning the junctions between the insert and the flanking regions for events MON 87427, MON 87460, MON 89034, MIR162 and NK603 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 87460 × MON 89034 × MIR162 × NK603 is highly unlikely.

In order to assess the possibility for horizontal gene transfer (HGT) by homologous recombination (HR), the applicant performed a sequence identity analysis to microbial DNA for events MON 87427, MON 87460, MON 89034, MIR162 and NK603. 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[8]

The GMO Panel assessed the applicant's literature searches on maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603, which include a scoping review, according to the guidelines given in EFSA (2010, 2017a).

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-2016-134. Based on the outcome of the scoping review, the GMO Panel agrees that there is limited value of undertaking a systematic review for maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 at present.

Although the overall quality of the performed literature searches is acceptable, the GMO Panel considers that future searches on maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 should 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;

[7] Dossier. Part II – Sections 1.2.1.3 and 1.2.2.2; additional information on 9/6/2017, 22/11/2018 and 30/4/2019.
[8] Dossier. Part II – Section 1.2.1.3.; additional information on 9/6/2017, 22/11/2018 and 30/4/2019.

None of the relevant publications identified through the literature searches reported information pointing to safety issues associated with the intended uses of maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 and its subcombinations.

3.4. Risk assessment of the five-event stack maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603

3.4.1. Molecular characterisation

In line with the requirements laid down by regulation (EU) 503/2013, the possible impact of the combination of the events on their integrity, the expression levels of the newly expressed proteins and the biological functions conferred by the individual inserts are considered below.

3.4.1.1. Genetic elements and their biological function

Maize events MON 87427, MON 87460, MON 89034, MIR162 and NK603 were combined by conventional crossing to produce MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603. The structure of the inserts introduced into maize MON 87427, MON 87460, MON 89034, MIR162 and NK603 is described in detail in the respective EFSA scientific opinions (Table 2) and no new genetic modifications were involved. Genetic elements in the expression cassettes of the single events are summarised in Table 3.

Intended effects of the inserts in maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 are summarised in Table 4.

Based on the known biological function of the newly expressed proteins (Table 4), the only foreseen interactions at the biological level are between the Cry proteins or between the Vip3Aa20 and the Cry proteins in susceptible insects.

Table 3: Genetic elements in the expression cassettes of the events stacked in maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
MON 87427	35S (CaMV)	–	CTP2 (*Arabidopsis thaliana*)	CP4 *epsps* (*Agrobacterium* sp.)	*nos* (*Agrobacterium tumefaciens*)
MON 87460	*act1* promoter (*Oryza sativa*)	*act1* leader and intron (*O. sativa*)	–	*CSPB* (*Bacillus subtilis*)	3′UTR of T-tr7 (*A. tumefaciens*)
	35S (CaMV)	–	–	*NPTII* (*Escherichia coli*)	*nos* (*A. tumefaciens*)
MON 89034	35S (CaMV)	CAB (*Triticum* sp.)	–	*cry1A.105* (*Bacillus thuringiensis*)	*Hsp17* (*Triticum* sp.)
	35S (FMV)	–	CTP (*Z. mays*)	*cry2Ab2* (*B. thuringiensis*)	*nos* (*A. tumefaciens*)
MIR162	ZmUbiInt (*Zea mays*)	–	–	*vip3Aa20* (*B. thuringiensis*)	35S (CaMV)
	ZmUbiInt (*Z. mays*)	–	–	*pmi* (*E. coli*)	*nos* (*A. tumefaciens*)
NK603	*act1* (*O. sativa*)	*act1* (*O. sativa*)	CTP2 (*A. thaliana*)	CP4 *epsps* (*Agrobacterium* sp.)	*nos* (*A. tumefaciens*)
	35S (CaMV)	I-*Hsp70* (*Z. mays*)	CTP2 (*A. thaliana*)	CP4 *epsps l214p* (*Agrobacterium* sp.)	*nos* (*A. tumefaciens*)

CaMV: cauliflower mosaic virus; 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 87460 × MON 89034 × MIR162 × NK603

Event	Protein	Donor organism and biological function	Intended effects in GM plant
MON 87427	CP4 EPSPS	Based on a gene from *Agrobacterium* strain CP4 (Barry et al., 2001). 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) is an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Herrmann, 1995)	Event MON 87427 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 87460	CSPB	Based on a gene from *Bacillus subtilis*. The cold shock protein B (CSPB) protein is an RNA chaperone associated with enhanced abiotic stress tolerance in bacteria (Phadtare et al., 2002a,b; Castiglioni et al., 2008)	Event MON 87460 expresses the bacterial CSPB protein. The *CspB* coding sequence is translated into the CSPB-L2V protein, which differs from the *B. subtilis* CSPB protein by one leucine-to-valine substitution at amino acid position 2. CSPB expression helps to reduce yield loss caused by drought stress
	NPTII	Based on a gene from bacterial transposon Tn5. Neomycin phosphotransferase II (NPTII) inactivates by phosphorylation a range of antibiotics, including kanamycin and neomycin (Fraley et al., 1983)	Event MON 87460 expresses the bacterial NPTII protein. NPTII was used as a marker to facilitate the selection process of transformed plant cells
MON 89034	Cry1A.105	Based on genes from *Bacillus thuringiensis* subsp. *kurstaki* and subsp. *aizawai*. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (cry) genes (Schnepf et al., 1998; Ellis et al., 2002)	Event 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 *Bacillus 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 protein, a protein toxic to certain lepidopteran larvae feeding on maize
MIR162	Vip3Aa20	Based on a gene from *Bacillus 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 (Vip) (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*. The phosphomannose isomerase (PMI) enzyme catalyses 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)

Event	Protein	Donor organism and biological function	Intended effects in GM plant
NK603	CP4 EPSPS	Based on a gene from *Agrobacterium* strain CP4 (Barry et al., 2001). 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) is an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Herrmann, 1995)	Event NK603 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
	CP4 EPSPS L214P	Based on a gene from *Agrobacterium* strain CP4 (Barry et al., 2001). 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) is an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Herrmann, 1995)	Event NK603 expresses also CP4 EPSPS L214P – this variant, compared to the CP4 EPSPS protein, contains a single amino acid substitution from leucine to proline at position 214. The two CP4 EPSPS protein variants are structurally and functionally equivalent

3.4.1.2. Integrity of the events in the five-event stack maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603[9]

The genetic stability of the inserted DNA over multiple generations in the single maize events MON 87427, MON 87460, MON 89034, MIR162 and NK603 was demonstrated previously (see Table 2). Integrity of these events in maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 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 five-event maize stack are identical to the sequences originally reported for the five single events, thus confirming that the integrity of these events was maintained in the five-event stack maize.

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

CP4 EPSPS, CSPB, NPTII, Cry1A.105, Cry2Ab2 Vip3Aa20 and PMI protein levels were analysed by enzyme-linked immunosorbent assay (ELISA) in material harvested in a field trial across five locations in the USA in the 2014 growing season. Samples analysed included leaf (V3–V4), grain (R6), root (V3–V4) and forage (R5) 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 the events, protein levels were determined for the five-event stack and the corresponding single events in different parts of the plant.

The levels of all the newly expressed proteins in the five-event stack and the corresponding singles were similar in all tissues, except for the expected difference in the CP4 EPSPS protein levels resulting from the combination of single events MON 87427 and NK603 both producing CP4 EPSPS protein in the five-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.

3.4.1.4. Conclusions of the molecular characterisation

The molecular data establish that the events stacked in maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins are similar in the five-stack maize and in the single events except for the expected higher level of CP4 EPSPS in the stack. 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.

Based on the known biological function of the newly expressed proteins, interactions at the biological level are expected between the Cry proteins or between the Vip3Aa20 and the Cry proteins in susceptible insects, which will be dealt with in Section 3.4.4. In addition, the potential impact of the RNA chaperon CSPB protein on the levels of the other newly expressed proteins was assessed by analysing the protein expression levels in the five-event stack and the respective singles. No impact of CSPB protein on the expression levels of the other newly expressed proteins was found.

[9] Dossier: Part II—Section 1.2.2.2
[10] Dossier: Part II – Section 1.2.2.3 and additional information: 7/8/2017 and 11/3/2019

3.4.2. Comparative analysis[11]

3.4.2.1. Overview of studies conducted for comparative analysis

Application EFSA-GMO-NL-2016-134 presents data on agronomic and phenotypic characteristics and on forage and grain composition of the five-event stack maize (Table 5).

Table 5: Overview of the comparative analysis studies to characterise the five-event stack maize provided in application EFSA-GMO-NL-2016-134

Study focus	Study details	Comparator	Non-GM reference varieties
Agronomic and phenotypic analysis	Field study, USA, 2014, eight sites(a)	MPA640B	17(b)
Compositional analysis	Field study, USA, 2014, eight sites(c)		18(b)

GM: genetically modified.
(a): The field trials were located in Jefferson, IA; Vermilion, IL; Warren, IL; Shelby, IL; Pawnee, KS; Perquimans, NC; Miami, OH and Berks, PA.
(b): Non-GM maize hybrids used in the 2014 field trials were Channel 211-97, Channel 213-88, Dekalb DKC62-06, Dekalb DKC63-43, Gateway 6158, LG2540, LG2548, Midland Phillips, Mycogen 2H721, Mycogen 2J790, NC + 5220, NH6280, NH6769, Phillips 717, Seed Consultants 1112, Stewart S588, Stewart S602 and Stine 9724. All the non-GM hybrids were used for both the agronomic and phenotypic characterisation and the compositional analyses except the non-GM hybrid maize Stewart S588 that was used for the compositional analysis only.
(c): The field sites were located in Jefferson, IA; Webster, IA; Champaign, IL; Warren, IL; Shelby, IL; Pawnee, KS; Miami, OH; and Berks, PA.

3.4.2.2. Experimental field trial design and statistical analysis

At each site, the following materials were grown: the five-event stack maize, the comparator maize MPA640B and four commercial non-GM maize reference varieties (hereafter 'non-GM reference varieties'). All materials were treated with conventional herbicides management regimes; in addition, the field trials included the five-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 (EFSA GMO Panel, 2010a,b, 2011a). This includes, for each of the two treatments of the five-event stack maize, the application of a difference test (between the GM stack maize and its non-GM comparator) and an equivalence test (between the GM stack maize and the set of non-GM reference varieties).[12] The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).[13]

3.4.2.3. Suitability of selected test materials

Selection of the GM maize line and comparator

To produce the five-event stack maize, the single events MON 87427, MON 87460, MON 89034, MIR162 and NK603 were transferred in the genetic background of two different non-GM inbred lines, LH244 and LH287.

In subsequent subsections, GM maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 refers to hybrid (F_1 generation) obtained crossing GM inbred line LH244 (carrying MIR162) with GM inbred line LH287 (carrying MON 87427 × MON 87460 × MON 89034 × NK603).

The comparator selected in the field trials is the hybrid maize MPA640B that was obtained by crossing the non-GM inbred lines LH244 and LH287. As documented by the pedigree, the GMO Panel considers the produced comparator acceptable for the comparative analysis.

The five-event stack maize and the non-GM comparator, both with a comparative relative maturity (CRM) of 110, are appropriate for growing in a range of environments across North America.

[11] Dossier: Part II – Section 1.3; additional information: 24/4/2017 and 7/8/2017.
[12] The purpose of the test of equivalence is to evaluate the estimated mean values for maize MON 87427 × MON 89034 × MIR162 × NK603 taking into account natural variability as defined by a set of non-GM reference varieties with a history of safe use for consumption as food or feed.
[13] 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).

Selection of non-GM reference varieties

The non-GM reference varieties (see Table 5) with a CRM ranging from 108 to 115 were selected by the applicant and at each selected site four of them were tested. On the basis of the information provided on CRM classes, the GMO Panel considers the selected non-GM reference varieties appropriate for the comparative assessment.

Seed production and quality

The seeds of the five-event stack maize and the comparator used in the 2014 field trials (see Table 5) were produced, harvested and stored under similar conditions. The seed lots were verified for their identity via event specific PCR analysis. The mean germination rates of the five-event stack maize and the comparator were 100% and 99%, respectively. The GMO Panel considers that the starting seed used as test material in the agronomic, phenotypic and compositional studies was of suitable quality.

Conclusion on suitability

The GMO Panel is of the opinion that the five-event stack maize, the non-GM comparator and the non-GM reference varieties were properly selected and are of adequate quality. Therefore, the test materials are considered appropriate 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.[14] The soil characteristics of the selected fields were diverse,[15] corresponding to optimal, near-optimal and sub-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.

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 dataset falls within the range of climatic conditions normally occurring at these sites.

Management practices

The field trials included plots containing five-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 five-event stack maize managed following the same agricultural practices, plus exposed to the intended glyphosate-containing herbicide. Glyphosate was applied at the V2–V4 growth stage. Despite not considered a normal agricultural practice, thinning was applied at all field trial sites to achieve a more homogeneous plant density across plots. The GMO Panel considers that the management practices, including sowing, harvesting and application of plant protection products, were acceptable for the field trials.

Conclusion on representativeness

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

3.4.2.5. Agronomic and phenotypic endpoints

Thirteen agronomic and phenotypic endpoints,[16] plus information on abiotic stressors, disease incidence and arthropod damage, were collected from eight different sites (see Table 5).

[14] For event MON 87460, a comparative analysis was specifically conducted under drought conditions (Table 2). Considering that there is no indication of an interaction between the events (see Section 3.4.1.4), it was not necessary to request the inclusion of field trials under drought conditions for the five-event stack maize.

[15] Soil types of the field trials were silty clay loam, loam, silt loam and sandy loam; soil organic matter ranged from 1.6% to 5.4%.

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

The endpoint dropped ears was not subjected to a formal statistical analysis (Section 3.4.2.2) because more than 90% of the values were 0.

The results of the statistical analysis were the following:

- For maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 (treated with conventional herbicides), the test of difference identified statistically significant differences with the comparator for six endpoints.[17] All these endpoints fell under equivalence category I or II, except for final stand count for which the test of equivalence was not applied (because the variation between the non-GM reference varieties was estimated to be 0).[18]
- For maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 (treated with the intended herbicides), the test of difference identified statistically significant differences with the comparator for eight endpoints.[19] All these endpoints fell under equivalence category I or II, except for days to 50% silking which fell under equivalence category III and final stand count for which the test of equivalence was not applied.[20]

The GMO Panel considered the changes observed for the five-event stack maize with respect to the non-GM comparator in final stand count (~1 plant/plot increase) and days to 50% silking (~1.5 days increase). Taking into account the magnitude of the differences and the results observed for the other endpoints, the GMO Panel considered that these differences do not affect the use of the field trials for the comparative analysis. Whether the differences can lead to an environmental adverse effect is considered in Section 3.5.

3.4.2.6. Compositional analysis

Forage and grain harvested from the field trials in the US in 2014 (Table 5) were analysed for 78 different constituents (nine in forage and 69 in grain), including the key constituents recommended by the OECD (2002). For 15 grain components,[21] more than 50% of the observations were below the limit of quantification.

The statistical analysis was applied to a total of 63 constituents (9 in forage[22] and 54 in grain[23]); a summary of the outcome of the test of difference and the test of equivalence is presented in Table 6:

- For maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 treated with the intended herbicide were identified 39 endpoints with statistically significant differences with its non-GM comparator. All the endpoints fell under equivalence category I and II.
- For maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 treated with the conventional herbicide, statistically significant differences with its non-GM comparator were identified for 46 endpoints. All the endpoints fell under equivalence category I and II. Moisture levels in forage fell under equivalence category III, although no statistically significant differences were identified with the non-GM comparator.

[17] Ear height, days to 50% pollen shed, plant height, days to 50% silking, final stand count and stalk lodged plants.

[18] Estimated mean values for final stand count were 67.9 (non-treated GM maize), 67.7 (treated GM maize), 66.8 (non-GM comparator) and 67.6 (non-GM reference varieties).

[19] Early stand count, grain moisture, days to 50% pollen shed, plant height, root lodged plants, days to 50% silking, final stand count, stalk lodged plants and yield.

[20] Estimated mean values for final stand count were 67.9 (non-treated GM maize), 67.7 (treated GM maize), 66.8 (non-GM comparator) and 67.6 (non-GM reference varieties). Estimated mean values for days to 50% silking were 66.0 (treated GM maize), 64.7 (non-GM comparator) and 63.7 (non-GM reference varieties); equivalence limits: (61.7, 65.8).

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

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

[23] Proximates (moisture, protein, total fat, ash, carbohydrates by calculation), fibre fractions (acid detergent fibre (ADF), neutral detergent fibre (NDF), total detergent fibre (TDF)), amino acids (alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine), fatty acids (palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C20:1), behenic acid (C22:0)) , vitamins (vitamin A (β-carotene), vitamin B1 (thiamine) , vitamin B2 , vitamin B6 , vitamin E (α-tocopherol), niacin and folic acid) , minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium and zinc) and other compounds (phytic acid, raffinose, ferulic acid and p-coumaric acid).

Table 6: Outcome of the comparative compositional analysis in seeds and forage for maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603. The table shows the number of endpoints in each category.

		Test of difference[a]			
		Treated[c]		Not-treated[c]	
		Not different	Significantly different	Not different	Significantly different
Test of equivalence[b]	Category I/II	21	39[d]	13	46[d]
	Category III/IV	–	–	1[e]	–
	Not categorised	3[f]	–	3[f]	–
	Total endpoints	63		63	

(a): Comparison between for maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 and its non-GM comparator.
(b): Four different outcomes: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence). Not categorised means that the test of equivalence was not applied because of the lack of variation among the non-GM reference varieties.
(c): Treated/not-treated with intended herbicide glyphosate.
(d): Endpoints with significant differences between maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 and its non-GM comparator falling in equivalence category I-II (treated and not-treated). For grain, both treated and not treated: carbohydrates by calculation, alanine, arginine, aspartic acid, glutamic acid, glycine, isoleucine, lysine, phenylalanine,proline, serine, threonine, tryptophan, tyrosine, valine, palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), eicosenoic acid (C20:1), total fat, folic acid, niacin, vitamin B1, vitamin B6, vitamin E, total dietary fibre (TDF), manganese, magnesium, potassium, zinc, ferulic acid, *p*-coumaric acid, and raffinose. Not treated only: protein, cystine/cysteine, histidine, leucine, arachidic acid (C20:0), vitamin A, phytic acid; treated only: phosphorus. For forage, both treated and not treated: protein and carbohydrates by calculation; only not treated: phosphorus
(e): Endpoints falling under equivalence category III/IV although no statistically significant differences were identified with respect to the non-GM comparator: moisture in forage (not-treated).
(f): Endpoints not categorised for equivalence and without significant differences between the MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 and its non-GM comparator: neutral detergent fibre (NDF), acid detergent fibre (ADF) and total fat in forage (treated and not treated).

The GMO Panel assessed all the compositional differences between maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 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 reference varieties. No endpoints showing significant differences between the five-event stack maize and the non-GM comparator and falling under category III/IV were identified.

3.4.2.7. Conclusion on the comparative assessment

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 the agronomic and phenotypic characteristics tested between the five-event stack maize and the non-GM comparator needs further assessment for environmental safety, except for the changes in days to 50% silking and final stand count which are considered in Section 3.4.4.1.
- None of the differences identified in forage and seed composition between the five-stack maize and the non-GM comparator needs further assessment regarding food and feed safety.

3.4.3. Food and feed safety assessment

3.4.3.1. Effects of processing

Maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 will undergo existing production processes used for conventional maize. No novel production process is envisaged. Based on the outcome of the comparative assessment, processing of the five-event stack maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 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

The effects of temperature and pH on the newly expressed proteins in this five-event stack maize have been previously evaluated by the GMO Panel (Table 2). No new information has been provided in the context of this application.

3.4.3.3. Toxicology

Testing of newly expressed proteins

Seven proteins (Cry1A.105, Cry2Ab2, Vip3Aa20, PMI, CSPB, NPTII, CP4 EPSPS and its variant CP4 EPSPS L214P) are newly expressed in maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 (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 87460 × MON 89034 × MIR162 × NK603 has been assessed with regard to human and animal health. The insecticidal proteins Cry1A.105 and Cry2Ab2 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 CSPB protein is an RNA chaperone associated with enhanced abiotic stress tolerance in bacteria and plants, through its interaction with RNA secondary structures, limiting their misfolding and allowing cells to maintain cellular functions under various stress conditions (Phadtare et al., 2002a,b; Castiglioni et al., 2008). The NPTII protein inactivates by phosphorylation a range of antibiotics (Fraley et al., 1983). 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 87460 × MON 89034 × MIR162 × NK603.

In vitro protein degradation studies on Cry1A.105, Cry2Ab2, Vip3Aa20, PMI, CSPB, NPTII, CP4 EPSPS and its variant CP4 EPSPS L214P proteins have been previously evaluated by the GMO Panel (Table 2). No new information has been provided in the context of this application.

The GMO Panel concludes that there are no safety concerns to human and animal health related to the newly expressed proteins Cry1A.105, Cry2Ab2, Vip3Aa20, PMI, CSPB, NPTII CP4 EPSPS and its variant CP4 EPSPS L214P protein in maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603.

Testing of new constituents other than proteins

No new constituents other than newly expressed proteins have been identified in maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603. Therefore, no further food and feed safety assessment of components other than the newly expressed proteins is required.

Information on altered levels of food and feed constituents

The five-event stack maize did not show any compositional differences to the non-GM comparator that would require further assessment (Section 3.4.2.6).

Testing of the whole genetically modified food and feed

Based on the outcome of the molecular characterisation assessment, 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 the five-event stack maize have been identified (see Sections 3.4.1., 3.4.2.3 and 3.4.3.2). Therefore, animal studies on food/feed derived from maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 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 87460, MON 89034, MIR162 and NK603. The five studies had already been provided in the context of the single-event applications and assessed by the GMO Panel; no adverse effects related to the administration of the respective GM diets had been identified (Table 2). In the context of the assessment of maize MON

87427 × MON 87460 × MON 89034 × MIR162 × NK603 and in order to fulfil the requirements of Regulation (EU) No 503/2013, the applicant provided additional information upon EFSA's request for the studies on the single-event maize MON 87427, MON 87460, MON 89034, MIR162 and NK603.

The GMO Panel has previously assessed the above-mentioned additional information on MON 87427, MON 89034, MIR162 and NK603 in the context of another application under Regulation (EU) 503/2013 (EFSA GMO Panel, 2019). The additional histopathology[24] provided for the 90-day study on maize MON 87460 showed sporadic histopathological findings compatible with the spontaneous background pathology of rats of this strain and age.

The GMO Panel concludes that these studies are in line with the legal requirements and confirms that there are no indications of adverse effects related to the 90-day administration to rats of diets including grains from maize MON 87427, MON 89034, MON 87460, NK603 and MIR162.

The GMO Panel noted that the incorporation rate of maize selected in these studies is up to 41.5%, in line with commercially available rodent diets. It has been recently reported that a diet incorporating 50% maize may be tolerated without inducing nutritional imbalances in rats after 90-day administration (Steinberg et al., 2019), but the GMO Panel considers that further scientific confirmation is needed before this 50% maize incorporation rate is applicable in future studies.

3.4.3.4. Allergenicity

For the allergenicity assessment, a weight-of-evidence approach was followed, taking into account all the information obtained on the newly expressed proteins, as no single piece of information or experimental method yields sufficient evidence to predict allergenicity (Codex Alimentarius, 2009; EFSA GMO Panel, 2011a; Regulation 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. In addition, an assessment of specific newly expressed proteins in relation to their potential to cause celiac disease was also performed (EFSA GMO Panel, 2017a).

Assessment of allergenicity of newly expressed proteins

For allergenicity, the GMO Panel has previously evaluated the safety of the proteins Cry1A.105, Cry2Ab2, Vip3Aa20, PMI, CSPB, NPTII and CP4 EPSPS (including its variant CP4 EPSPS L214P) proteins individually, and no concerns on allergenicity were identified in the context of the applications assessed (Table 2). No new information on allergenicity of these proteins that might change the previous conclusions of the GMO Panel in the context of the GM events assessed has become available.[25] Based on the current knowledge, and as none of the newly expressed proteins showed allergenicity, no reasons for concerns regarding the simultaneous presence of these newly expressed proteins in this five-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 on Cry1Ac when applied at relatively high doses (e.g. Vázquez et al., 1999). The GMO Panel has previously evaluated the safety of the Cry1A.105, Cry2Ab2 and Vip3Aa20 proteins and no concerns on adjuvanticity in the context of the applications assessed were identified (Table 2). The levels of Bt proteins in this five-event stack maize are comparable to those in the respective single maize events (Section 3.4.1.4). From the limited experimental evidence available, the GMO Panel did not find indications that the presence of the Bt proteins at the levels expressed in this five-event stack maize might act as adjuvants with the potential to enhance a specific immunoglobulin E (IgE) response and to favour the development of an allergic reaction.

The applicant provided spontaneous information on the safety of the Cry1A.105, Cry2Ab2, CSPB, NPTII and CP4 EPSPS proteins regarding their potential hazard to cause a celiac disease response. For such assessment, the applicant followed the principles described in the EFSA GMO Panel guidance document (2017). Briefly, bioinformatics searches for sequence identity with proteins eliciting celiac disease revealed partial matches with and without the Q/E-X1-P-X2 motif for the Cry1A.105 protein requiring further

[24] Aorta, bone (sternum) with bone marrow, cecum, cervix (females only), eyes with optic nerves, lung (including bronchi), mandibular lymph node, Peyer's Patches, skin with mammary gland (females only), skin from males (similar area), esophagus, pituitary, prostate (males only), mandibular salivary gland, seminal vesicles (males only), skeletal muscle, trachea, urinary bladder, uterus (females only), and vagina (females only) from all animals given the control and 33% test diet.

[25] Information on the safety of the Cry1A.105, Cry2Ab2, CspB, NptII and CP4 EPSPS proteins regarding their potential hazard to cause a celiac disease response has been spontaneously submitted by the applicant despite 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'.

investigation. Based on additional considerations on position and nature of amino acids flanking the QLPQ motif, such as the absence of prolines at specific positions and the charge and size of adjacent amino acids (EFSA GMO Panel, 2017a), the sequences containing the motif do not raise concern as they fail to mimic gluten sequences. Two additional partial sequence matches lacking the motif were also identified and subjected to a HLA-DQ-peptide structure modelling using a publicly available crystal structure of HLA-DQ2-T-cell receptor interactions as a reference.[26,27] These two sequences were associated to a potential gamma-gliadin-derived peptide, for which the 9-amino acid core sequence has not been determined and which lacks a proven clinical relevance[28] (Tye-Din et al., 2010). For this reason, these two sequences are not considered relevant. Therefore, no indications of safety concerns were identified by the GMO Panel. Finally, it is acknowledged that the platform used by the applicant for the modelling is in line with the recommendations by EFSA (EFSA GMO Panel, 2017b). However, in general, it will be also necessary to perform direct comparisons between the sequence(s) under question and the matching clinically relevant celiac disease T-cell epitope(s) included in the pertinent HLA-DQ crystal structure.

Assessment of allergenicity of the 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[29] (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 (Sections 3.4.1, 3.4.2 and 3.4.3), the GMO Panel identified no indications of a potentially increased allergenicity of food and feed derived from this five-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, CSPB, NPTII, Cry1A.105, Cry2Ab2, Vip3Aa20, and PMI proteins newly expressed in MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 maize. Dietary exposure was estimated based on protein expression levels reported in this application for the five-event stack maize treated with the intended herbicide, the current available consumption data and feed practices, the foods and feeds currently available on the market and the described processing conditions.

Table 7 describes the protein expression levels used to estimate both human and animal dietary exposure.

Table 7: Mean values (n = 20, μg/g dry weight and μg/g fresh weight) for newly expressed proteins in grains and forage from MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 maize treated with the intended herbicide[(a)]

Protein	Tissue/developmental stage	
	Grains/R6 (μg/g dry weight and μg/g fresh weight)	Forage/R5 (μg/g dry weight)
CP4 EPSPS[(b)]	15.0/13	210
CSPB	0.082/0.074	0.079
NPTII	0.0063/0.0057[(c)]	0.18
Cry1A.105	8.1/7.3	26
Cry2Ab2	1.6/1.5	34
Vip3Aa20	38/33[(d)]	69
PMI	1.2/1.1[(d)]	4

EPSPS: 5-enolpyruvylshikimate-3-phosphate synthase; CSPB: cold shock protein B; NPTII: neomycin phosphotransferase II protein; PMI: phosphomannose isomerase; LOQ: limit of quantification.

(a): Intended herbicide: glyphosate.

(b): CP4 EPSPS levels in MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 maize are the sum of two protein variants, CP4 EPSPS (expressed in MON 87427 and NK603) and CP4 EPSPS L214P (expressed in NK603).

(c): N = 6 since fourteen samples were reported as below the LOQ (LOQ = 0.005 μg/g fw).

(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 dry weight correction factor of 0.88 to account for approximately 12% moisture content in the grains.

[26] https://www.rcsb.org/

[27] https://www.rosettacommons.org/software

[28] This aspect points out the importance of developing comprehensive database regarding celiac disease epitopes that is appropriately built, curated regularly and designed for risk assessment purposes.

Human dietary exposure[29]

Dietary exposure was estimated across different European countries on different population groups: young population (infants, toddlers, 'other children'), adult population (adolescents, 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 87460 × MON 89034 × MIR162 × NK603 maize grains were derived from replicated field trials (four replicates from five locations) in the 2014 US growing season. Mean values (fresh weight) are considered as the most adequate to estimate dietary exposure (see Table 7). Since no specific consumption data were available on commodities containing, consisting of or obtained from MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 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).[30] 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 assigned to the processed commodities the mean value reported for the newly expressed proteins in maize grains. This is a conservative approach as neither recipes nor the effect of processing is considered on the final concentration of newly expressed proteins. Summary statistics from the EFSA consumption database were used.[31] 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[32] among consumers only and those exposures derived from the mean consumption of the remaining food categories in the total population (EFSA, 2015). Table 8 shows the highest acute dietary exposure for the different newly expressed proteins; dietary exposure estimates ranged between 0.02 μg/kg body weight (bw) per day for NPTII in adults (18–65 years) and 268 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).

Table 8: Highest acute dietary exposure to CP4 EPSPS, CSPB, NPTII, Cry1A.105, Cry2Ab2, Vip3Aa20, and PMI 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[(a)]	**CSPB**	**NPTII**	**Cry1A.105**	**Cry2Ab2**	**Vip3Aa20**	**PMI**
Toddlers	106	0.6	0.05	59	12	268	8.9
Adults	46	0.3	0.02	26	5.3	117	3.9

bw: body weight; EPSPS: 5-enolpyruvylshikimate-3-phosphate synthase; CSPB: cold shock protein B; NPTII: neomycin phosphotransferase II protein; PMI: phosphomannose isomerase.

(a): CP4 EPSPS levels in MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 maize are the sum of two protein variants, CP4 EPSPS (expressed in MON 87427 and NK603) and CP4 EPSPS L214P (expressed in NK603).

The GMO Panel estimated chronic dietary exposure to CP4 EPSPS, CSPB, NPTII, Cry1A.105, Cry2Ab2, Vip3Aa20 and PMI proteins. Individual consumption data of the relevant food commodities were retrieved from the EFSA Consumption Database, using dietary surveys with at least two days consumption and covering a total of 22 European countries.[33] Different recipes and factors were considered to estimate the amount of maize in the consumed commodities before assigning CP4 EPSPS, CSPB, NPTII, Cry1A.105, Cry2Ab2, Vip3Aa20 and PMI proteins levels to the relevant commodities.[34] No losses in the newly expressed proteins during processing were considered, except for certain commodities excluded from the exposure estimations (maize oil, corn starch, corn syrup). The 95th

[29] Dossier: Part II – Section 2.4.

[30] http://www.efsa.europa.eu/en/data/food-consumption-data

[31] Summary statistics from the EFSA Comprehensive European Food Consumption Database accessed in September 2016.

[32] Dominant food commodity refers to the food that will lead to the highest exposure among all consumed foods.

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

[34] Example: 100 g 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 29.7 μg of Vip3Aa20 per gram of maize bread as compared to 33 μg/g in the maize grains.

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

	N	Chronic dietary exposure (μg/kg bw per day)						
		CP4 EPSPS[(a)]	CSPB	NPTII	Cry1A.105	Cry2Ab2	Vip3Aa20	PMI
Infants	11	0–58.4	0–0.3	0–0.03	0–32.8	0–6.7	0–148.4	0–4.9
Toddlers	14	3.2–54.4	0.02–0.3	0.001–0.02	1.8–30.5	0.4–6.3	8.1–138.0	0.3–4.6
Other children	19	8.6–47.6	0.05–0.3	0.004–0.02	4.8–26.8	1.0–5.5	21.8–120.9	0.7–4.0
Adolescents	18	1.9–35.7	0.01–0.2	0.001–0.002	1.0–20.0	0.2–4.1	4.7–90.6	0.2–3.0
Adults	19	0.9–17.9	0.005–0.1	0.0004–0.01	0.5–10.1	0.1–2.1	2.2–45.5	0.1–1.5
Elderly and very elderly	18	0.1–11.0	0.001–0.1	0.00005–0.005	0.1–6.2	0.01–1.3	0.3–28.0	0.01–0.9
Special population[(b)]	4	5.3–26.0	0.03–0.1	0.002–0.01	3.0–14.6	0.6–3.0	13.4–66.0	0.4–2.2

bw: body weight; n: number of dietary surveys; EPSPS: 5-enolpyruvylshikimate-3-phosphate synthase; CSPB: cold shock protein B; NPTII: neomycin phosphotransferase II protein; PMI: phosphomannose isomerase.
(a): CP4 EPSPS levels in MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 maize are the sum of two protein variants, CP4 EPSPS (expressed in MON 87427 and NK603) and CP4 EPSPS L214P (expressed in NK603).
(b): Pregnant women and lactating women.

Table 9 shows the chronic dietary exposure to each of the newly expressed proteins across European dietary surveys; dietary exposure ranged between 0.00005 μg/kg bw per day for NPTII protein in elderly and very elderly population (> 65 years) and 148.4 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'.

Animal dietary exposure[35]

Animal dietary exposure to CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20, CSPB, NPTII and PMI 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 CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20, CSPB, NPTII and PMI proteins in maize grains and forage/silage were derived from field trials conduct in the 2014 US growing season (see Table 7). To estimate the mean newly expressed proteins 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 newly expressed proteins occur during processing.

Dietary exposure to CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20, PMI, CSPB and NPTII proteins in maize MON 87427 × MON 87460 x MON 89034 × MIR162 × NK603 following the consumption of maize grain, gluten feed and gluten meal was provided by the applicant across different animal species (i.e. broiler, finishing pig and lactating 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 as follows:

- to CP4 EPSPS protein, 1,768 μg/kg bw per day in broiler chickens, 1,442 μg/kg bw per day in dairy cattle and 869 μg/kg bw per day in finishing pig.
- to Cry1A.105 protein, 955 μg/kg bw per day in broiler chickens, 779 μg/kg bw per day in dairy cattle and 469 μg/kg bw per day in finishing pig.
- to Cry2Ab2 protein, 189 μg/kg bw per day in broiler chickens, 154 μg/kg bw per day in dairy cattle and 93 μg/kg bw per day in finishing pig.
- to Vip3Aa20 protein, 4,480 μg/kg bw per day in broiler chickens, 3,654 μg/kg bw per day in dairy cattle and 2,200 μg/kg bw per day in finishing pig.

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- to PMI protein, 141 μg/kg bw per day in broiler chickens, 115 μg/kg bw per day in dairy cattle and 69 μg/kg bw per day in finishing pig.
- to CSPB protein, 9.7 μg/kg bw per day in broiler chickens, 7.9 μg/kg bw per day in dairy cattle and 4.7 μg/kg bw per day in finishing pig.
- to NPTII protein, 0.7 μg/kg bw per day in broiler chickens, 0.6 μg/kg bw per day in dairy cattle and 0.4 μg/kg bw per day in finishing pig.

The GMO Panel estimated dietary exposure to CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20, CSPB, NPTII and PMI proteins in maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 across different livestock animal species (beef and dairy cows, lamb and breeding swine) 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 was as follows:

- to CP4 EPSPS protein, 4,032 μg/kg bw per day in beef, 4,846 μg/kg bw per day in dairy cow, 2,677 μg/kg bw per day in lamb, 969 μg/kg bw per day in breeding swine and 1,436 μg/kg bw per day in layer hen.
- to Cry1A.105 protein, 500 μg/kg bw per day in beef, 600 μg/kg bw per day in dairy cow, 331 μg/kg bw per day in lamb, 120 μg/kg bw per day in breeding swine and 178 μg/kg bw per day in layer hen.
- to Cry2Ab2 protein, 653 μg/kg bw per day in beef, 785 μg/kg bw per day in dairy cow, 433 μg/kg bw per day in lamb, 157 μg/kg bw per day in breeding swine and 233 μg/kg bw per day in layer hen.
- to Vip3Aa20 protein, 1,324 μg/kg bw per day in beef, 1,592 μg/kg bw per day in dairy cow, 880 μg/kg bw per day in lamb, 318 μg/kg bw per day in breeding swine and 472 μg/kg bw per day in layer hen.
- to PMI protein, 77 μg/kg bw per day in beef, 92 μg/kg bw per day in dairy cow, 51 μg/kg bw per day in lamb, 18 μg/kg bw per day in breeding swine and 27 μg/kg bw per day in layer hen.
- to CSPB protein, 1.5 μg/kg bw per day in beef, 1.8 μg/kg bw per day in dairy cow, 1 μg/kg bw per day in lamb, 0.3 μg/kg bw per day in breeding swine and 0.5 μg/kg bw per day in layer hen.
- to NPTII protein, 3.4 μg/kg bw per day in beef, 4.1 μg/kg bw per day in dairy cow, 2.3 μg/kg bw per day in lamb, 0.8 μg/kg bw per day in breeding swine and 1.2 μg/kg bw per day in layer hen.

3.4.3.6. Nutritional assessment of endogenous constituents

The intended traits of the five-event stack maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 are herbicide- and drought tolerance and insect resistance, with no intention to alter nutritional parameters. Comparison of the composition of maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 with the non-GM comparator and non-GM reference varieties did not identify differences that would require further safety assessment. From these data, the GMO Panel concludes that the nutritional impact of maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603-derived food and feed is the same as that expected from the non-GM comparator and non-GM reference varieties.

3.4.3.7. Conclusion of the food and feed safety assessment

The proteins CP4 EPSPS, CSPB, NPTII, Cry1A.105, Cry2Ab2, Vip3Aa20 and PMI newly expressed in the five-event stack maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 do not raise safety concerns for human and animal health. Interactions between these newly expressed proteins raising food and feed safety concerns (toxicological, allergenicity and adjuvanticity) are not expected. The nutritional impact of the five-event stack maize foods and feeds is expected to be the same as those from the comparator and non-GM reference varieties. The GMO Panel concludes that the five-event stack maize, as described in this application, is as safe as and nutritionally equivalent to the non-GM comparator and the non-GM reference varieties tested.

3.4.4. Environmental risk assessment[36]

Considering the scope of application EFSA-GMO-NL-2016-134, which excludes cultivation, the environmental risk assessment (ERA) of maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 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 87460 × MON 89034 × MIR162 × NK603 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 87460 × MON 89034 × MIR162 × NK603 and the observed differences in days to 50% silking and final stand count (see Section 3.4.2.5) will provide a selective advantage to maize plants, except when they are exposed to glyphosate-containing herbicides, under drought-stress or infested by insect pests that are susceptible to the Cry1A.105, Cry2Ab2 and/or Vip3Aa20 proteins.

The GMO Panel considers that the fitness advantage provided by the intended traits, and the observed differences in days to 50% silking and final stand count (see Section 3.4.2.5) will not allow the GM plant to overcome other biological and abiotic factors (described above) limiting plant's persistence and invasiveness. Therefore, the presence of the intended traits and other observed differences will not affect the persistence and invasiveness of the GM plant.

In conclusion, the GMO Panel considers it very unlikely that maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 will differ from conventional maize hybrid varieties in its ability to survive until subsequent seasons, or to establish occasional feral plants under European environmental conditions in case of accidental release into the environment of viable maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 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 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 as a result of an unlikely, but theoretically possible, HGT of the recombinant genes to bacteria in the gut of domesticated animals and humans fed GM material or other receiving environments was identified. The applicant submitted updated bioinformatic analysis for each of the single events in order to assess the possibility for HGT by HR.

The updated bioinformatic analyses provided in this application for the events MON 87427, MON 89034, MIR162 and NK603 have recently been assessed by the GMO Panel in the context of other applications (EFSA GMO Panel, 2019). The GMO Panel concluded that the unlikely, but

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theoretically possible, horizontal transfer of recombinant genes from these maize events to bacteria did not raise any environmental safety concern.

For the event MON 87460, the probability and potential adverse effects of HGT of the recombinant DNA was assessed by the GMO Panel in 2012 (EFSA GMO Panel, 2012a). Three scenarios for HGT were analysed: (1) the mobilisation of *npt*II by the cre/lox system, (2) transfer of *npt*II by double HR to a Ti-plasmid of *A. tumefaciens*, and (3) substitutive HR of *npt*II or *csp*B genes to the bacteria harbouring natural variants of such genes. The GMO Panel considered that the stabilisation of the *loxP-npt*II-*loxP* fragment due to the Cre recombination system present in bacteria containing a P1 or P1-like bacteriophage was unlikely.

Updated bioinformatic analysis for MON 87460 did not result in new information which would change previous conclusions on possible HGT as described in the three scenarios above. There is sufficient sequence identity and length of the *npt*II gene with bacterial DNA for HR but not for the codon-optimised *csp*B gene from *B. subtilis*. Other genetic elements with sequence identity to bacterial DNA are the T-tr7 intervening sequence upstream of the *npt*II, and the left border of the Ti cassette downstream of the *npt*II which were already considered for facilitating HGT. Double HR between these sequences and the corresponding sequences in the *A. tumefaciens* Ti-plasmid would result in the insertion of the *npt*II expression cassette (*P35S/npt*II*/T-nos)* and the concomitant loss of a naturally occurring Ti-plasmid sequence resulting in a Ti-plasmid that would not promote for plant tumour formation (EFSA GMO Panel, 2012a). Due to the selective disadvantage of such bacterial recipients for growing in plants, and the natural abundance of *npt*II genes in the environmental bacterial communities, the GMO Panel concludes that there was no indication for a risk to human or animal health or to the environment.

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

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

Plant-to-plant gene transfer

The potential for occasional feral maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 plants originating from grain import spills to transfer recombinant DNA to sexually compatible plants and the environmental consequences of this transfer were considered.

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

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-2016-134 into account (no cultivation), potential interactions of occasional feral maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 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 maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 grains is limited and because ingested proteins are degraded before entering the

environment through faecal material of animals fed GM maize, potential interactions of maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 with non-target organisms are not considered by the GMO Panel to raise any relevant environmental safety concern. Interactions that may occur between the *Bt* proteins 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 maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 plants arising from grain import spills is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions with the abiotic environment and biogeochemical cycles are not considered 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 maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 would differ from conventional maize varieties in its ability to persist under European environmental conditions. Considering the scope of application EFSA-GMO-NL-2016-134, interactions of occasional feral maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from the five-event stack maize to bacteria does not indicate a safety concern. Therefore, considering the combined traits and their interactions, the outcome of the comparative analysis, the routes and levels of exposure, the GMO Panel concludes that maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 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 five-event stack maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603

No new data on the five single maize events MON 87427, MON 87460, MON 89034, MIR162 and NK603 that would lead to a modification of the original conclusions on their safety were identified.

The combination of maize events MON 87427, MON 87460, MON 89034, MIR162 and NK603 in the five-event stack maize did not give rise to issues concerning the molecular, agronomic/phenotypic or compositional characteristics of the five-event stack maize that would be of concern for food and feed safety and nutrition.

The newly expressed proteins in the five-event 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 87460 × MON 89034 × MIR162 × NK603. Comparison of the levels of the newly expressed proteins between the five-event stack maize and those of the single maize events did not reveal an interaction at protein expression level.

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 87460 × MON 89034 × MIR162 × NK603 would not raise safety concerns in the event of accidental release of viable GM maize grains into the environment.

No scientific information that could change the conclusions on this five-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 87460 × MON 89034 × MIR162 × NK603, 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[37]

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.[38] In this case, the risk assessment takes as its starting point the assessment of the single maize events, and uses the data generated for the five-

[37] Additional information: 24/5/2018.
[38] 115th GMO Panel meeting (Annex 1 of the minutes: http://www.efsa.europa.eu/sites/default/files/event/170517-m.pdf).

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 eleven subcombinations and no safety concerns were identified: the two-event maize stack MON 89034 × NK603; the three-event stack maize MON 87427 × MON 89034 × NK603 and all its subcombinations; the four-event stack maize MON 87427 × MON 89034 × MIR162 × NK603 and all its subcombinations (see Table 2). Literature searches covering the 10 years before submission of the application (January 2006–October 2016) 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.[39] Consequently, the GMO Panel considers that its previous conclusions on these subcombinations remain valid.

3.5.2. Subcombinations not previously assessed

Out of 25 subcombinations included in the scope of this application, 14 have not been previously assessed by the GMO Panel, and no experimental data were provided for these maize stacks (see Table 10).

Table 10: Maize stacks not previously assessed and covered by the scope of application EFSA-GMO-NL-2016-134

Degree of stacking	Events
Four-event stack	MON 89034 × MON 87460 × MIR162 × MON 87427
	NK603 × MON 87460 × MIR162 × MON 87427
	NK603 × MON 89034 × MON 87460 × MON 87427
	NK603 × MON 89034 × MON 87460 × MIR162
Three-event stack	MON 87460 × MIR162 × MON 87427
	MON 89034 × MON 87460 × MON 87427
	MON 89034 × MON 87460 × MIR162
	NK603 × MON 87460 × MON 87427
	NK603 × MON 87460 × MIR162
	NK603 × MON 89034 × MON 87460
Two-event stack	MON 87460 × MON 87427
	MON 87460 × MIR162
	MON 89034 × MON 87460
	NK603 × MON 87460

3.5.2.1. Stability of the events

The genetic stability of the inserted DNA over multiple generations in the five single maize events was demonstrated previously (see Table 2). Integrity of the events was demonstrated in the five-event stack maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 (Section 3.4.1.2) and the previously assessed maize subcombinations (EFSA GMO Panel, 2009, 2017a, 2019). The GMO Panel finds no reasons to expect the loss of integrity of the events in the maize subcombinations not previously assessed (see Table 10).

3.5.2.2. Expression of the events

The GMO Panel assessed whether any combination of the five events by conventional crossing could result in significant changes in expression levels of the newly expressed proteins, as this could indicate an unexpected interaction between the events. Based on current knowledge of the molecular elements introduced, there is no reason to expect interactions that would affect the levels of the newly expressed proteins in the 14 subcombinations compared with those in the single maize events. This assumption was confirmed by comparing the levels of the newly expressed proteins of each single maize event with those of the five-event stack maize. The levels were similar in the five-event stack

[39] Dossier: Part II – Section 7; additional information: 13/8/2018; 15/11/2018.

maize and in the single events except for CP4 EPSPS, which showed, in general, the expected higher level in the stack resulting from the combination of the single events MON 87427 and NK603 (Section 3.4.1.3 and Appendix A). Therefore, there was no indication of an interaction at protein expression level. In addition, expression data from the two-event stack maize MON 89034 × NK603 (EFSA GMO Panel, 2009), the three-event stack MON 87427 × MON 89034 × NK603 (EFSA GMO Panel, 2017a) and the four-event stack MON 87427 × MON 89034 × MIR162 × NK603 (EFSA GMO Panel, 2019) were similar to those observed in each of the single maize events or showed in general the expected higher levels for CP4 EPSPS. This supports the conclusion that interactions affecting the expression levels of the newly expressed proteins are not expected in the 14 subcombinations not previously assessed and included in the scope of application EFSA-GMO-NL-2016-134.

3.5.2.3. Potential functional interactions between the events

The GMO Panel assessed the potential for interactions between maize events in the 14 subcombinations not previously assessed (Table 10), taking into consideration intended traits and unintended effects.

Based on the known biological functions of the individual newly expressed proteins (Table 4), there is currently no expectation for possible interactions relevant for the food and feed or environmental safety between these proteins in those subcombinations. The GMO Panel took into account all the intended and potential unintended effects considered in the assessment of the five single events, the previously assessed subcombinations (Table 2) and the five-event stack maize. It is concluded that none of these 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 five-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 14 subcombinations included in the scope of application EFSA-GMO-NL-2016-134, 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 five-event stack maize.

3.6. Post-market monitoring[40]

3.6.1. Post-market monitoring of GM food/feed

The GMO Panel concluded that the five-event stack maize, as described in this application, is nutritionally equivalent to and as safe as the non-GM comparator and the non-GM reference varieties tested (Section 3.4.3.7). Eleven of the subcombinations have been previously assessed and no safety concerns were identified. The 14 subcombinations not previously assessed and included in the scope of application EFSA-GMO-NL-2016-134 are expected to be as safe as and nutritionally equivalent to the single maize events, the previously assessed maize subcombinations and the five-event stack maize (Section 3.5.3). Therefore, the GMO Panel considers that post-market monitoring of food and feed from the five-event stack maize and its subcombinations, as described in this application, is not necessary.

3.6.2. Post-market environmental monitoring

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

[40] Dossier: Part II – Sections 4 and 6.

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

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

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

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

In the context of annual PMEM reports, the applicant could further fine–tune future literature searches according to the GMO Panel recommendations given in Section 3.3.

3.6.3. Conclusion 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 87460 × MON 89034 × MIR162 × NK603.

4. Overall conclusions

The GMO Panel was asked to carry out a scientific assessment of maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 and subcombinations for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003.

No new information on the five single maize events MON 87427, MON 87460, MON 89034, MIR162 and NK603 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 in the five-event stack maize does not give rise to food/feed safety and nutritional concerns. The GMO Panel concludes that the five-event stack maize, as described in this application, is as safe as and nutritionally equivalent to its non-GM comparator and the non-GM reference varieties tested.

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

Since no new data on the eleven 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 14 subcombinations included in the scope of application EFSA-GMO-NL-2016-134, no information has been provided. The GMO Panel assessed possible interactions between the events in the 14 subcombinations, and concludes that these combinations of events MON 87427, MON 87460, MON 89034, MIR162 and NK603 would not raise safety concerns. These subcombinations are therefore expected to be as safe as and nutritionally equivalent to the maize single events, the previously assessed subcombinations and the five-event stack maize.

Based on the relevant publications identified through the literature searches, the GMO Panel did not identify any safety issues pertaining to the intended uses of maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 and its subcombinations. In the context of annual PMEM reports, the applicant could further fine–tune 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 five-event stack maize and its subcombinations.

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

necessary. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of the five-event stack maize and its subcombinations.

In conclusion, the GMO Panel considers that maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 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 03 November 2016 concerning a request for authorisation of the placing on the market of maize MON87427 × MON87460 × MON89034 × MIR162 × NK603 (EFSA-GMO-NL-2016-134) submitted in accordance with Regulation (EC) No 1829/2003 by Monsanto Europe S.A./N.V.
- Application EFSA-GMO-NL-2016-134 validated by EFSA, 19 January 2017
- Request for supplementary information to the applicant, 24 January 2017
- Receipt of supplementary information from the applicant, 30 January 2017
- Request for supplementary information to the applicant, 24 February 2017
- Request for supplementary information to the applicant, 12 April 2017
- Receipt of supplementary information from the applicant, 24 April 2017
- Request for supplementary information to the applicant, 06 June 2017
- Receipt of supplementary information from the applicant, 09 June 2017
- Request for supplementary information to the applicant, 06 July 2017
- Receipt of supplementary information from the applicant, 14 July 2017
- Receipt of supplementary information from the applicant, 07 August 2017
- Request for supplementary information to the applicant, 15 February 2018
- Request for supplementary information to the applicant, 23 March 2018
- Receipt of supplementary information from the applicant, 24 May 2018
- Request for supplementary information to the applicant, 18 June 2018
- Receipt of supplementary information from the applicant, 13 August 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, 08 November 2018
- Receipt of supplementary information from the applicant, 16 November 2018
- Receipt of supplementary information from the applicant, 22 November 2018
- Request for supplementary information to the applicant, 21 December 2018
- Request for supplementary information to the applicant, 25 January 2019
- Request for supplementary information to the applicant, 07 February 2019
- Receipt of supplementary information from the applicant, 11 March 2019
- Receipt of supplementary information from the applicant, 19 March 2019
- Receipt of supplementary information from the applicant, 27 March 2019
- Request for supplementary information to the applicant, 24 April 2019
- Receipt of supplementary information from the applicant, 30 April 2019
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- Receipt of supplementary information from the applicant, 21 May 2019

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Abbreviations

ADF	acid detergent fibre
bw	body weight
CaMV	cauliflower mosaic virus
CSPB	cold shock protein B
CRM	comparative relative maturity
CTP	chloroplast transit peptide
ELISA	enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	environmental risk assessment
FMV	Figwort Mosaic Virus
fw	fresh weight
GM	genetically modified
GMO	genetically modified organism
GMO Panel	EFSA Panel on Genetically Modified Organisms
HGT	horizontal gene transfer
HR	homologous recombination

IgE	immunoglobulin E
LOQ	limit of quantification
NDF	neutral detergent fibre
NPTII	neomycin phosphotransferase II protein
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PCR	polymerase chain reaction
PMEM	post-market environmental monitoring
PMI	phosphomannose isomerase
T-DNA	transfer-deoxyribonucleic acid
UTR	untranslated region

Appendix A – Protein expression data

Means, standard deviation and ranges of protein levels (μg/g dry weight) from maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 (treated with glyphosate), MON 87427 (treated with glyphosate), MON 87460 (not treated), MON 89034 (not treated), MIR162 (not treated) and NK603 (treated with glyphosate), from field trials performed in USA in 2014[a]

Protein	Event(s)	Leaf (V3-V4)	Root (V3-V4)	Forage (R5)	Grain (R6)
CP4 EPSPS[b]	**MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603**	1,100[c] ±180[d] (810–1,400)[e]	340 ± 54 (250–420)	210 ± 68 (99–290)	15 ± 2.2 (12–20)
	MON 87427	800 ± 92 (620–940)	240 ± 47 (170–330)	150 ± 57 (51–240)	7.3 ± 1.3 (5.2–9.5)
	NK603	280 ± 56 (200–380)	130 ± 25 (66–170)	66 ± 26 (28–130)	9.7 ± 1.9 (6.5–14)
CSPB	**MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603**	2.0 ± 0.46 (1.3–3.3)	1.2 ± 0.40 (0.57–2.1)	0.079 ± 0.022 (0.054–0.13)	0.082 ± 0.019 (0.056–0.12)
	MON 87460	2.1 ± 0.52 (1.1–2.8)	1.5 ± 0.55 (0.35–2.2)	0.092 ± 0.020 (0.061–0.14)	0.082 ± 0.018 (0.060–0.12)
NPTII	**MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603**	4.7 ± 0.99 (3.1–6.5)	0.86 ± 0.14 (0.61–1.2)	0.18 ± 0.054 (0.071–0.28)	0.0063 ± 0.00091 (0.0055–0.0080)
	MON 87460	4.3 ± 1.1 (3.0–6.5)	0.91 ± 0.22 (0.57–1.5)	0.19 ± 0.046 (0.078–0.28)	0.006 ± 0.00045 (0.0056–0.0065)
Cry1A.105	**MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603**	850 ± 350 (250–1,300)	61 ± 11 (43–83)	26 ± 6.4 (10–36)	8.1 ± 3.5 (3.8–15)
	MON 89034	880 ± 220 (270–1,300)	70 ± 15 (49–100)	26 ± 6.6 (16–37)	7.2 ± 3.7 (4.1–16)
Cry2Ab2	**MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603**	170 ± 46 (120–310)	160 ± 37 (85–210)	34 ± 8.7 (17–49)	1.6 ± 0.40 (1.0–2.4)
	MON 89034	150 ± 25 (110–180)	160 ± 44 (74–250)	28 ± 5.8 (20–38)	1.9 ± 0.39 (1.3–2.8)
Vip3Aa20	**MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603**	130 ± 35 (79–220)	50 ± 7.8 (35–65)	69 ± 20 (33–110)	38 ± 6.2 (29–50)
	MIR162	130 ± 34 (74–200)	57 ± 7.4 (44–68)	68 ± 22 (44–120)	38 ± 5.6 (29–49)
PMI	**MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603**	11 ± 3.0 (6.9–17)	6.8 ± 1.2 (4.5–9.6)	4.0 ± 2.0 (0.90–9.7)	1.2 ± 0.16 (0.94–1.5)
	MIR162	11 ± 2.3 (8.4–15)	7.2 ± 1.5 (5.0–11)	3.6 ± 1.3 (1.7–5.7)	1.4 ± 0.30 (0.82–2.0)

(a): Number of sample is n = 19 or n = 20 except for: n = 18 for forage/R5 (for Cry1A.105 and Cry2Ab2 in MON 89034); n = 6 and n = 3 for grain/R6 (for NPTII in the five-event stack and MON 87460, respectively).
(b): EPSPS levels in the maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 are a sum of two protein variants, CP4 EPSPS (expressed in MON 87427 and NK603) and CP4 EPSPS L214P (expressed in NK603).
(c): Mean.
(d): Standard deviation.
(e): Range.

Appendix B – 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 87460 × MON 89034 × MIR162 × NK603 for humans, animal or the environment

Study identification	Title
MSL0026336	Southern Blot Analyses to Confirm the Presence of MON 87427, MON 87460, MON 89034, and NK603 in the Combined Trait Maize Product MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603
MSL0026337	Southern Blot Analyses to Confirm the Presence of MIR162 in the Combined Trait Maize Product MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603
MSL0026638	Compositional Analyses of Maize Grain from MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 Grown in the United States in 2014
MSL0026879	Phenotypic Evaluation and Environmental Interactions of Maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 in 2014 U.S. Field Trials
MSL0026880	Phenotypic Evaluation of Maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 with Herbicide Treatment in 2014 U.S. Field Trials
MSL0027322	An Evaluation of the Potential for Interaction between MON 87460 and the Insecticidal Traits in the Combined Maize Product MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 with Corn Earworm (*Helicoverpa zea*)
MSL0027523	Comparison of Lipid Transfer Protein (LTP) Expression Levels from MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 with Conventional Control Maize