Assessment of genetically modified maize MON 87427 x MON 87460 x MON 89034 x 1507 x MON 87411 x 59122 and subcombinations, for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2017-139)

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SCIENTIFIC OPINION

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Abstract

Maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 (six-event stack maize) was produced by conventional crossing to combine six single events: MON 87427, MON 87460, MON 89034, 1507, MON 87411 and 59122. The GMO Panel previously assessed the six single maize events and 17 of the subcombinations and did not identify safety concerns. No new data on the single maize events or the 17 subcombinations were identified that could lead to modification of the original conclusions on their safety. The molecular characterisation, comparative analysis (agronomic, phenotypic and compositional characteristics) and the outcome of the toxicological, allergenicity and nutritional assessment indicate that the combination of the single maize events and of the newly expressed proteins and dsRNA in the six-event stack maize does not give rise to food and feed safety and nutritional concerns. The GMO Panel concludes that the six-event stack maize, as described in this application, is as safe as its non-GM comparator and the selected non-GM reference varieties. In the case of accidental release of viable grains of the six-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 39 maize subcombinations not previously assessed and concludes that these are expected to be as safe as the single events, the previously assessed subcombinations and the six–event stack maize. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of the six-event stack maize. Post-market monitoring of food/feed is not considered necessary. The GMO Panel concludes that the six-event stack maize and its subcombinations are as safe as the non-GM comparator and the selected non-GM reference varieties with respect to potential effects on human and animal health and the environment.

Keywords: dsRNA, GMO, herbicide tolerant, *Zea mays*, insect resistant, stack events

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Question number: EFSA-Q-2017-00115

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Summary

Following the submission of application EFSA-GMO-NL-2017-139 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 (GM) glufosinate and glyphosate tolerant, insect resistant and drought tolerant maize (*Zea mays* L.) MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 (referred to hereafter as 'six-event stack maize') and its subcombinations independently of their origin, according to Regulation (EU) No 503/2013 (referred to hereafter as 'subcombinations'). The scope of application EFSA-GMO-NL-2017-139 is for import, processing, and food and feed uses within the European Union (EU) of maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 and all its subcombinations independently of their origin, and does not include cultivation in the EU.

The term 'subcombination' refers to any combination of up to five of the events present in the six-event stack maize. The safety of subcombinations occurring as segregating progeny in the harvested grains of maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 is evaluated in the context of the assessment of the six-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 six-event stack, are risk assessed separately in the present scientific opinion.

The six-event stack maize was produced by conventional crossing to combine six single maize events: MON 87427 expressing the 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein to confer tolerance to glyphosate-containing herbicides; MON 87460 expressing the cold shock protein B (CspB) (to confer drought tolerance) and the neomycin phosphotransferase II protein (NPTII) (used as a selectable marker); MON 89034 expressing the Cry1A.105 and Cry2Ab2 proteins (for protection against certain lepidopteran pests); 1507 expressing the Cry1F protein (for protection against certain lepidopteran pests) and the PAT protein (for tolerance to glufosinate-ammonium-containing herbicides); MON 87411 expressing the Cry3Bb1 protein and the DvSnf7 dsRNA (for protection against certain coleopteran pests) and the CP4 EPSPS protein (for tolerance to glyphosate-containing herbicides); and 59122 expressing the Cry34Ab1 and Cry35Ab1 proteins (for protection against certain coleopteran pests) and the PAT protein.

The GMO Panel evaluated the six-event stack maize and its subcombinations with reference to the scope and appropriate principles described in its applicable guidelines for the risk assessment of GM plants and the post-market environmental monitoring. The GMO Panel considered the information submitted in application EFSA-GMO-NL-2017-139, additional information provided by the applicant during the risk assessment, the scientific comments submitted by the Member States and the relevant scientific literature.

For application EFSA-GMO-NL-2017-139, previous assessments of the six single events (MON 87427, MON 87460, MON 89034, 1507, MON 87411 and 59122), and 17 of the subcombinations provided a basis for the assessment of the six-event stack maize and all its subcombinations. No safety concerns were identified by the GMO Panel in the previous assessments. No safety issue concerning the six 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 six-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 carried out, and the safety of the newly expressed proteins, of the dsRNAs and the whole food and feed were evaluated with respect to potential toxicity, allergenicity and nutritional characteristics. Environmental impacts and post-market environmental monitoring (PMEM) plan were also evaluated.

The molecular characterisation data establish that the events stacked in maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 have retained their integrity. Protein expression analysis showed that the levels of the newly expressed proteins are similar in the six-event stack maize and in the single events, except for CP4 EPSPS and PAT protein levels that are expected to be different because of the combination of events MON 87427 and MON 87411 (both producing CP4 EPSPS) and events 1507 and 59122 (both producing PAT) in the in the six-event stack maize. In addition, the provided data indicate that there is no impact of the dsRNAs on the expression

levels of the newly expressed proteins. No indications were identified of interactions that may affect the integrity of the events and the levels of the newly expressed proteins in this six–event stack maize.

The comparative analysis of agronomic and phenotypic characteristics and grain and forage composition identified no differences between maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 and the non-GM comparator that required further assessment except for the changes in root lodged plants, in levels of acid detergent fibre (ADF) in forage and in levels of protein, arginine, glycine, leucine, lysine and manganese in grain. These changes were further assessed for food/feed safety and environmental impact and raised no concern.

The molecular characterisation, the comparative analysis and the outcome of the toxicological, allergenicity and nutritional assessment indicate that the combination of the single maize events and of the newly expressed proteins and dsRNA in the six-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 × 1507 × MON 87411 × 59122, as described in this application, is as safe as the non-GM comparator and the selected commercial non-GM maize reference varieties (referred to hereafter as non-GM reference varieties).

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 × 1507 × MON 87411 × 59122 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 17 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 39 subcombinations included in the scope of application EFSA-GMO-NL-2017-139, no experimental data were provided. The GMO Panel assessed the possibility of interactions between the events in the 39 subcombinations and concludes that these subcombinations would not raise safety concerns. These subcombinations are therefore expected to be as safe as the single events, the previously assessed subcombinations and the six-event stack maize.

Given the absence of safety concerns for foods and feeds from maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 and its subcombinations, the GMO Panel considers that post–market monitoring of these products is not necessary. The PMEM plan and reporting intervals are in line with the intended uses of the six-event stack maize and its subcombinations. The literature searches did not identify any relevant publications on maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122. In the context of annual PMEM reports, the applicant could further fine tune future literature searches according to the GMO Panel recommendations provided in this scientific opinion.

The GMO Panel concludes that maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 and its subcombinations, as described in this application, are as safe as the non-GM comparator and the selected non-GM reference varieties with respect to potential effects on human and animal health and the environment.

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1. Introduction

The scope of application EFSA-GMO-NL-2017-139 is for food and feed uses, import and processing within the European Union (EU) of the genetically modified (GM) herbicide-tolerant, insect-resistant and drought-tolerant maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 and all its subcombinations independently of their origin and does not include cultivation in EU.

1.1. Background

On 21 February 2017, the European Food Safety Authority (EFSA) received from the Competent Authority of The Netherlands application EFSA-GMO-NL-2017-139 for authorisation of maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 (hereafter referred to as 'the six-event stack maize') (Unique Identifier MON-87427-7 × MON-8746Ø-4 × MON-89Ø34-3 × DAS-Ø15Ø7-1 × MON-87411-9 × DAS-59122-7), submitted by Monsanto Europe S.A. (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003[1].

Following receipt of application EFSA-GMO-NL-2017-139, 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 31 May 2017, EFSA declared the application valid and made the 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-2017-139. 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 MS 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-2017-139 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 × 1507 × MON 87411 × 59122 and all its subcombinations independently of their origin according to the of application EFSA-GMO-NL-2017-139.

According to Regulation (EC) No 1829/2003, this scientific opinion is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation including the opinions of the nominated risk assessment bodies of EU Member States.[5]

In addition to the present scientific opinion, EFSA and its GMO Panel were also asked to report on the particulars listed under Articles 6(5) and 18(5) of Regulation (EC) No 1829/2003. The relevant information is made available in the EFSA Register of Questions,[2] including the information required under Annex II to the Cartagena Protocol, a labelling proposal, a post-market environmental monitoring (PMEM) plan as provided by the applicant; the methods, validated by the Community

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.

[2] Available online at the EFSA Register of Questions: http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2017-00115

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

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

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

reference laboratory, for detection, including sampling, identification of the transformation events in the food-feed and/or foods-feeds produced from it and the appropriate reference materials.

2. Data and methodologies

2.1. Data

The GMO Panel based its scientific assessment of maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 on the valid application EFSA-GMO-NL-2017-139, additional information provided by the applicant during the risk assessment, relevant scientific comments submitted by EU MS and relevant peer-reviewed scientific publications. As part of this comprehensive information package, the GMO Panel received additional unpublished studies submitted by the applicant in order to comply with the specific provisions of Regulation (EU) No 503/2013. A list of these additional unpublished studies is provided in Appendix A.

2.2. Methodologies

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

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

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

In the frame of the contracts OC/EFSA/GMO/2014/01 and OC/EFSA/GMO/2018/02 contractors performed preparatory work and delivered report on the methods applied by the applicant in performing statistical and toxicological analyses, respectively.

3. Assessment

3.1. Introduction

Application EFSA-GMO-NL-2017-139 covers the six-event stack maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 and all its 56 subcombinations independently of their origin (Table 1).

Table 1: Stacked maize events covered by the scope of application EFSA-GMO-NL-2017-139

Degree of stacking	Event	Unique identifiers
Six-event stack	MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122	MON-87427-7 × MON-8746Ø-4 × MON-89Ø34-3 × DAS-Ø15Ø7-1 × MON-87411-9 × DAS-59122-7
Five-event stack	59122 × MON 89034 × MON 87460 × MON 87427 × MON 87411	DAS-59122-7 × MON-89Ø34-3 × MON-8746Ø-4 × MON-87427-7 × MON-87411-9
	1507 × MON 89034 × MON 87460 × MON 87427 × MON 87411	DAS-Ø15Ø7-1 × MON-89Ø34-3 × MON-8746Ø-4 × MON-87427-7 × MON-87411-9
	1507 × 59122 × MON 87460 × MON 87427 × MON 87411	DAS-Ø15Ø7-1 × DAS-59122-7 × MON-8746Ø-4 × MON-87427-7 × MON-87411-9
	1507 × 59122 × MON 89034 × MON 87427 × MON 87411	DAS-Ø15Ø7-1 × DAS-59122-7 × MON-89Ø34-3 × MON-87427-7 × MON-87411-9
	1507 × 59122 × MON 89034 × MON 87460 × MON 87411	DAS-Ø15Ø7-1 × DAS-59122-7 × MON-89Ø34-3 × MON-8746Ø-4 × MON-87411-9
	1507 × 59122 × MON 89034 × MON 87460 × MON 87427	DAS-Ø15Ø7-1 × DAS-59122-7 × MON-89Ø34-3 × MON-8746Ø-4 × MON-87427-7

Degree of stacking	Event	Unique identifiers
Four-event stack	MON 89034 × MON 87460 × MON 87427 × MON 87411	MON-89Ø34-3 × MON-8746Ø-4 × MON-87427-7 × MON-87411-9
	59122 × MON 87460 × MON 87427 × MON 87411	DAS-59122-7 × MON-8746Ø-4 × MON-87427-7 × MON-87411-9
	59122 × MON 89034 × MON 87427 × MON 87411	DAS-59122-7 × MON-89Ø34-3 × MON-87427-7 × MON-87411-9
	59122 × MON 89034 × MON 87460 × MON 87411	DAS-59122-7 × MON-89Ø34-3 × MON-8746Ø-4 × MON-87411-9
	59122 × MON 89034 × MON 87460 × MON 87427	DAS-59122-7 × MON-89Ø34-3 × MON-8746Ø-4 × MON-87427-7
	1507 × MON 87460 × MON 87427 × MON 87411	DAS-Ø15Ø7-1 × MON-8746Ø-4 × MON-87427-7 × MON-87411-9
	1507 × MON 89034 × MON 87427 × MON 87411	DAS-Ø15Ø7-1 × MON-89Ø34-3 × MON-87427-7 × MON-87411-9
	1507 × MON 89034 × MON 87460 × MON 87411	DAS-Ø15Ø7-1 × MON-89Ø34-3 × MON-8746Ø-4 × MON-87411-9
	1507 × MON 89034 × MON 87460 × MON 87427	DAS-Ø15Ø7-1 × MON-89Ø34-3 × MON-8746Ø-4 × MON-87427-7
	1507 × 59122 × MON 87427 × MON 87411	DAS-Ø15Ø7-1 × DAS-59122-7 × MON-87427-7 × MON-87411-9
	1507 × 59122 × MON 87460 × MON 87411	DAS-Ø15Ø7-1 × DAS-59122-7 × MON-8746Ø-4 × MON-87411-9
	1507 × 59122 × MON 87460 × MON 87427	DAS-Ø15Ø7-1 × DAS-59122-7 × MON-8746Ø-4 × MON-87427-7
	1507 × 59122 × MON 89034 × MON 87411	DAS-Ø15Ø7-1 × DAS-59122-7 × MON-89Ø34-3 × MON-87411-9
	1507 × 59122 × MON 89034 × MON 87427	DAS-Ø15Ø7-1 × DAS-59122-7 × MON-89Ø34-3 × MON-87427-7
	1507 × 59122 × MON 89034 × MON 87460	DAS-Ø15Ø7-1 × DAS-59122-7 × MON-89Ø34-3 × MON-8746Ø-4
Three-event stack	MON 87460 × MON 87427 × MON 87411	MON-8746Ø-4 × MON-87427-7 × MON-87411-9
	MON 89034 × MON 87427 × MON 87411	MON-89Ø34-3 × MON-87427-7 × MON-87411-9
	MON 89034 × MON 87460 × MON 87411	MON-89Ø34-3 × MON-8746Ø-4 × MON-87411-9
	MON 89034 × MON 87460 × MON 87427	MON-89Ø34-3 × MON-8746Ø-4 × MON-87427-7
	59122 × MON 87427 × MON 87411	DAS-59122-7 × MON-87427-7 × MON-87411-9
	59122 × MON 87460 × MON 87411	DAS-59122-7 × MON-8746Ø-4 × MON-87411-9
	59122 × MON 87460 × MON 87427	DAS-59122-7 × MON-8746Ø-4 × MON-87427-7
	59122 × MON 89034 × MON 87411	DAS-59122-7 × MON-89Ø34-3 × MON-87411-9
	59122 × MON 89034 × MON 87427	DAS-59122-7 × MON-89Ø34-3 × MON-87427-7
	59122 × MON 89034 × MON 87460	DAS-59122-7 × MON-89Ø34-3 × MON-8746Ø-4
	1507 × MON 87427 × MON 87411	DAS-Ø15Ø7-1 × MON-87427-7 × MON-87411-9
	1507 × MON 87460 × MON 87411	DAS-Ø15Ø7-1 × MON-8746Ø-4 × MON-87411-9
	1507 × MON 87460 × MON 87427	DAS-Ø15Ø7-1 × MON-8746Ø-4 × MON-87427-7
	1507 × MON 89034 × MON 87411	DAS-Ø15Ø7-1 × MON-89Ø34-3 × MON-87411-9
	1507 × MON 89034 × MON 87427	DAS-Ø15Ø7-1 × MON-89Ø34-3 × MON-87427-7
	1507 × MON 89034 × MON 87460	DAS-Ø15Ø7-1 × MON-89Ø34-3 × MON-8746Ø-4
	1507 × 59122 × MON 87411	DAS-Ø15Ø7-1 × DAS-59122-7 × MON-87411-9
	1507 × 59122 × MON 87427	DAS-Ø15Ø7-1 × DAS-59122-7 × MON-87427-7
	1507 × 59122 × MON 87460	DAS-Ø15Ø7-1 × DAS-59122-7 × MON-8746Ø-4
	1507 × 59122 × MON 89034	DAS-Ø15Ø7-1 × DAS-59122-7 × MON-89Ø34-3

Degree of stacking	Event	Unique identifiers
Two-event stack	MON 87427 × MON 87411	MON-87427-7 × MON-87411-9
	MON 87460 × MON 87411	MON-8746Ø-4 × MON-87411-9
	MON 87460 × MON 87427	MON-8746Ø-4 × MON-87427-7
	MON 89034 × MON 87411	MON-89Ø34-3 × MON-87411-9
	MON 89034 × MON 87427	MON-89Ø34-3 × MON-87427-7
	MON 89034 × MON 87460	MON-89Ø34-3 × MON-8746Ø-4
	59122 × MON 87411	DAS-59122-7 × MON-87411-9
	59122 × MON 87427	DAS-59122-7 × MON-87427-7
	59122 × MON 87460	DAS-59122-7 × MON-8746Ø-4
	59122 × MON 89034	DAS-59122-7 × MON-89Ø34-3
	1507 × MON 87411	DAS-Ø15Ø7-1 × MON-87411-9
	1507 × MON 87427	DAS-Ø15Ø7-1 × MON-87427-7
	1507 × MON 87460	DAS-Ø15Ø7-1 × MON-8746Ø-4
	1507 × MON 89034	DAS-Ø15Ø7-1 × MON-89Ø34-3
	1507 × 59122	DAS-Ø15Ø7-1 × DAS-59122-7

The term 'subcombination' refers to any combination of up to five of the maize events MON 87427, MON 87460, MON 89034, 1507, MON 87411 and 59122.

The safety of subcombinations occurring as segregating progeny in harvested grains of maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 is evaluated in the context of the assessment of the six-event stack maize in Section 3.5 of the present scientific opinion.

'Subcombination' also covers combinations that have either been or could be produced by conventional crossing through targeted breeding approaches (EFSA GMO Panel, 2011a). These are maize stacks that can be bred, produced and marketed independently of the six-event stack maize. These subcombinations are assessed in Section 3.5 of this scientific opinion.

The six-event stack maize was produced by conventional crossing to combine six single maize events: MON 87427 expressing the 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein to confer tolerance to glyphosate-containing herbicides; MON 87460 expressing the cold shock protein B (CspB) to confer drought tolerance and expressing the neomycin phosphotransferase II protein (NPTII) used as selectable marker to facilitate the selection process of transformed plant cells; MON 89034 expressing the Cry1A.105 and Cry2Ab2 proteins to confer protection against certain lepidopteran pests; 1507 expressing the Cry1F protein to confer protection against certain lepidopteran pests and the PAT protein to confer tolerance to glufosinate-ammonium-containing herbicides; MON 87411 expressing the Cry3Bb1 protein and the DvSnf7 dsRNA to confer protection against certain coleopteran pests and the CP4 EPSPS protein for tolerance to glyphosate-containing herbicides; and 59122 expressing the Cry34Ab1 and Cry35Ab1 protein to confer protection against certain coleopteran pests and the PAT protein. It should be noted that the assessment of herbicide residues in maize herbicide-tolerant crops relevant for this application has been investigated by the EFSA Pesticides Unit (EFSA, 2018).

All 6 single maize events, 10 two-event stacks, 6 three-event stacks and a four-event stack maize 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 × 1507 × MON 87411 × 59122 previously assessed by the GMO Panel

Event	Application or mandate	EFSA Scientific Opinion
1507	EFSA-Q-2004-011	EFSA (2004)
	EFSA-GMO-NL-2004-02	EFSA (2005a)
	EFSA-Q-2006-00330	EFSA (2005b)
	EFSA-GMO-RX-1507	EFSA (2009a)
	EFSA-GMO-RX-001	EFSA GMO Panel (2017a)
59122	EFSA-GMO-NL-2005-12	EFSA (2007)
	EFSA-GMO-NL-2005-23	EFSA GMO Panel (2013)
	EFSA-GMO-RX-003	EFSA GMO Panel (2017b)
MON 89034	EFSA-GMO-NL-2007-37	EFSA (2008)
	EFSA-GMO-RX-015	EFSA GMO Panel (2019a)
MON 87460	EFSA-GMO-NL-2009-70	EFSA GMO Panel (2012)
MON 87427	EFSA-GMO-BE-2012-110	EFSA GMO Panel (2015b)
MON 87411	EFSA-GMO-NL-2015-124	EFSA GMO Panel (2018a)
1507 × 59122	EFSA-GMO-NL-2005-15	EFSA (2009b)
1507 × MON 89034	EFSA-GMO-CZ-2008-62	EFSA GMO Panel (2010c)
	EFSA-Q-2011-01132	EFSA GMO Panel (2011c)
1507 × MON 87427	EFSA-GMO-BE-2013-118	EFSA GMO Panel (2017c)
59122 × MON 89034	EFSA-GMO-CZ-2008-62	EFSA GMO Panel (2010c)
	EFSA-Q-2011-01132	EFSA GMO Panel (2011c)
59122 × MON 87427	EFSA-GMO-BE-2013-118	EFSA GMO Panel (2017c)
MON 89034 × MON 87460	EFSA-GMO-NL-2016-134	EFSA GMO Panel (2019b)
MON 89034 × MON 87427	EFSA-GMO-BE-2013-117	EFSA GMO Panel (2017d)
MON 89034 × MON 87411	EFSA-GMO-NL-2017-144	EFSA GMO Panel (2019c)
MON 87460 × MON 87427	EFSA-GMO-NL-2016-134	EFSA GMO Panel (2019b)
MON 87427 × MON 87411	EFSA-GMO-NL-2017-144	EFSA GMO Panel (2019c)
1507 × 59122 × MON 89034 and subcombinations	EFSA-GMO-CZ-2008-62	EFSA GMO Panel (2010c)
	EFSA-Q-2011-01132	EFSA GMO Panel (2011c)
1507 × 59122 × MON 87427 and subcombinations	EFSA-GMO-BE-2013-118	EFSA GMO Panel (2017c)
1507 × MON 89034 × MON 87427 and subcombinations	EFSA-GMO-BE-2013-118	EFSA GMO Panel (2017c)
59122 × MON 89034 × MON 87427 and subcombinations	EFSA-GMO-BE-2013-118	EFSA GMO Panel (2017c)
MON 89034 × MON 87460 × MON 87427 and subcombinations	EFSA-GMO-NL-2016-134	EFSA GMO Panel (2019b)
MON 89034 × MON 87427 × MON 87411 and subcombinations	EFSA-GMO-NL-2017-144	EFSA GMO Panel (2019c)
1507 × 59122 × MON 89034 × MON 87427 and subcombinations	EFSA-GMO-BE-2013-118	EFSA GMO Panel (2017c)

3.2. Updated information on single events

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

The applicant clarified that the 1507 maize sequence reported for the six-event stack maize contained one silent nucleotide change in the insert sequence compared to the corrected original 1507 maize sequence (EFSA GMO Panel, 2018b, 2018c, 2019d, 2019e). Analysis of the new sequencing data and bioinformatic analyses performed on the new sequence does not identify any need for further safety assessment (EFSA GMO Panel, 2017d). Analysis of the corrected sequencing data and the bioinformatic analyses performed on this sequence did not give rise to safety issues.

The applicant clarified that the maize 59122 sequence reported in this application corresponds to the sequence submitted in the original application EFSA-GMO-NL-2005-12 of the single event (EFSA, 2007), but corrected for sequencing errors affecting three single nucleotides.[6]

Bioinformatics analyses on the junction regions for maize events MON 87427, MON 87460, MON 89034, 1507, MON 87411 and 59122, using the most up-to-date nucleotide sequences and methodology specified in EFSA guidance (EFSA GMO Panel, 2011a), confirmed that no known endogenous genes were disrupted by any of the inserts.

Updated bioinformatics analyses of the amino acid sequence of the newly expressed CP4 EPSPS, CspB, NPTII, Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins confirm previous analyses indicating no significant similarities to toxins and 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, 1507, MON 87411 and 59122 confirmed outputs of previous analysis (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 × 1507 × MON 87411 × 59122 is highly unlikely.

According to Regulation (EU) No 503/2013, when silencing approaches by RNAi have been used in GM plant applications, a bioinformatic analysis to identify potential 'off target' genes is required. The applicant has followed the recommendations by the GMO Panel for an RNAi off-target search in the six-stack maize expressing the DvSnf7 dsRNA.[7,8] Updated bioinformatics analysis confirms previous results that do not indicate an off-target effect of the DvSnf7 dsRNA expression that would need further assessment.

In order to assess the possibility for horizontal gene transfer (HGT) by homologous recombination, the applicant performed a sequence identity analysis with microbial DNA for maize events MON 87427, MON 87460, MON 89034, 1507, MON 87411 and 59122. 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

The GMO Panel assessed the applicant's literature searches on maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122, which included a scoping review, according to the guidelines given in EFSA (2010, 2017a, 2019).

A systematic review as referred to in Regulation (EU) No 503/2013 has not been provided in support to the risk assessment of application EFSA-GMO-NL-2017-139. Based on the outcome of the scoping review, the GMO Panel agrees that there is limited value in undertaking a systematic review for maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 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 × 1507 × MON 87411 × 59122 could be fine-tuned. 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) and that enough truncation is used and used consistently.

The literature searches did not identify any relevant publications on maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122.

[6] Dossier: Part II – Section 1.2.2.2 and additional information 17/1/2019, 27/8/209 and 17/12/2019.
[7] Annex II of the minutes of the 118th GMO plenary meeting (https://www.efsa.europa.eu/sites/default/files/event/171025-m.pdf).
[8] Additional information 17/1/2019.

3.4. Risk assessment of the six-event stack maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122

3.4.1. Molecular characterisation[9]

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

3.4.1.1. Genetic elements and their biological function

Maize events MON 87427, MON 87460, MON 89034, 1507, MON 87411 and 59122 were combined by conventional crossing to produce the six–event stack maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122. The structures of the inserts introduced into the six-event stack maize are 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 × 1507 × MON 87411 × 59122 are summarised in Table 4. Based on the known biological function of the newly expressed proteins (Table 4), the only foreseen interactions at the biological level are between the six Cry proteins in susceptible insects, which will be dealt with in Sections 3.4.4.

Table 3: Genetic elements in the expression cassettes of the events stacked in maize 1507 × 59122 × MON 89034 × MON 87460 × MON 87427 × MON 87411

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
MON 87427	*e35S* (CaMV)	*hsp70*	CTP2 (*Arabidopsis thaliana*)	*cp4 epsps* (*Agrobacterium* sp.)	*nos* (*Agrobacterium tumefaciens*)
MON 87460	*rac1* (*Oryza sativa*)	*rac1* (*Oryza sativa*)	–	*cspB* (*Bacillus subtilis*)	*tr7* (*A. tumefaciens*)
	35S (*CaMV*)	–	–	*nptII* (*Escherichia coli*)	*nos* (*A. tumefaciens*)
MON 89034	*35S* (CaMV)	*cab* (*Triticum aestivum*)	–	*cry1A.105* (*Bacillus thuringiensis*)	*hsp17* (*Triticum aestivum*)
	35S (FMV)	*hsp70*	CTP (*Zea mays*)	*cry2Ab2* (*Bacillus thuringiensis*)	*nos* (*Agrobacterium tumefaciens*)
1507	*ubi*ZM1 (*Zea mays*)	–	–	*cry1F* (*Bacillus thuringiensis*)	ORF25PolyA (*Agrobacterium tumefaciens*)
	35S (CaMV)	–	–	*pat* (*Streptomyces viridochromogenes*)	*35S* (CaMV)
MON 87411	*35S* (CaMV)	*Hsp70*	–	*snf7* (*Diabrotica virgifera virgifera*)	E9 (*Pisum sativum*)
	pIIG (*Zea mays*)	*cab* (*Triticum aestivum*)	–	*cry3Bb1* (*Bacillus thuringiensis*)	*hsp17* (*Triticum aestivum*)
	tubA (*Oryza sativa*)	–	*CTP2* (*Arabidopsis thaliana*)	*cp4 epsps* (*Agrobacterium* sp.)	*tubA* (*Oryza sativa*)
59122	*ubi*ZM1 (*Zea mays*)	–	–	*cry34Ab1* (*Bacillus thuringiensis*)	*pin*II (*Solanum tuberosum*)

[9] Dossier: Part II – Section 1.2 and additional information 17/1/2019, 27/8/2019 and 17/12/2019.

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
	wheat peroxidase (*Triticum aestivum*)	–	–	*cry35Ab1* (*Bacillus thuringiensis*)	*pin*II (*Solanum tuberosum*)
	35S (CaMV)	–	–	*pat* (*Streptomyces viridochromogenes*)	*35S* (CaMV)

UTR: untranslated region.
–: when no element was specifically introduced to optimise expression.

Table 4: Characteristics and intended effects of the events stacked in maize 1507 × 59122 × MON 89034 × MON 87460 × MON 87427 × MON 87411

Event	Protein/ dsRNA	Donor organism and biological functions	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 aminoglycoside 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, a protein toxic to certain lepidopteran larvae feeding on maize
1507	Cry1F	Based on genes from *Bacillus thuringiensis* subsp. *aizawai*. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (cry) genes (Schnepf et al., 1998; Ellis et al., 2002)	Event 1507 expresses a truncated version of the Cry1F protein. Cry1F is a protein toxic to certain lepidopteran larvae feeding on maize
	PAT	Based on a gene from *Streptomyces viridochromogenes* Tü494.	Event 1507 expresses the PAT protein which confers tolerance to glufosinate-

Event	Protein/ dsRNA	Donor organism and biological functions	Intended effects in GM plant
		Phosphinothricin-acetyl-transferase (PAT) enzyme acetylates L-glufosinate-ammonium (Thompson et al., 1987; Wohlleben et al., 1988; Eckes et al., 1989)	ammonium-based herbicides (Droge-Laser et al., 1994)
MON 87411	DvSnf7 dsRNA	Based on genes from western corn rootworm (WCR) (*Diabrotica virgifera virgifera* LeConte). The full-length Snf7 protein is part of the intracellular protein trafficking pathway (ESCRT) which is important for the maintenance of a functional intracellular transport of transmembrane proteins (Baum et al., 2007; Ramaseshadri et al., 2013)	Event MON 87411 expresses DvSnf7 dsRNA which is a small RNA toxic to western corn rootworm feeding on maize
	Cry3Bb1	Based on genes from *Bacillus thuringiensis*. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes (Schnepf et al., 1998; Ellis et al., 2002)	Event MON 87411 expresses the Cry3Bb1, a protein toxic to certain coleopteran larvae feeding on maize
	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 87411 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
59122	Cry34Ab1	Based on genes from *Bacillus thuringiensis* strain PS149B1. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes (Schnepf et al., 1998; Ellis et al., 2002)	Event 59122 expresses the Cry34Ab1 and Cry35Ab1; in complex these proteins are toxic to certain coleopteran larvae feeding on maize
	Cry35Ab1	Based on genes from *Bacillus thuringiensis* strain PS149B1. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes (Schnepf et al., 1998; Ellis et al., 2002)	
	PAT	Based on a gene from *Streptomyces viridochromogenes* Tü494. Phosphinothricin-acetyl-transferase (PAT) enzyme acetylates L-glufosinate-ammonium (Thompson et al., 1987; Wohlleben et al., 1988; Eckes et al., 1989)	Event 59122 expresses the PAT protein which confers tolerance to glufosinate ammonium-based herbicides (Droge-Laser et al., 1994)

3.4.1.2. Integrity of the events in the six-event stack maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122

The genetic stability of the inserted DNA over multiple generations in the single maize events MON 87427, MON 87460, MON 89034, 1507, MON 87411 and 59122 was demonstrated previously (see Table 2 and Section 3.2). Integrity of these events in maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 was demonstrated by sequence analysis showing that the sequences of the events (inserts and their flanking regions) in the six-event maize stack are identical to the sequences already assessed (see Table 2 and Section 3.2) for the six single events, thus confirming that the integrity of these events was maintained in the six-event stack maize.

3.4.1.3. Information on the expression of the inserts

CP4 EPSPS, CspB, NPTII, Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1 and Cry35Ab1 protein levels were analysed by enzyme-linked immunosorbent assay (ELISA), in material harvested in a field trial across five locations in the USA in 2014. Samples analysed included leaf (V3-V4), root (V3-V4), forage (R5) and grain (R6) both those treated and not treated with glyphosate and/or glufosinate-ammonium. In order to assess changes in protein expression levels which may result from potential interactions between the events, protein levels were determined for the six-event maize stack and the corresponding single events in different parts of the plant.

The levels of all the proteins newly expressed in the six-event stack maize and the corresponding singles were comparable in all tissues, except for CP4 EPSPS and PAT protein levels that are expected to be different because of the combination of events MON 87427 and MON 87411 both producing CP4 EPSPS and events 1507 and 59122 both producing PAT protein in the six-event stack maize (Appendix B). In addition, the potential impact of the DvSnf7 dsRNA on the levels of the newly expressed proteins was assessed by comparing the protein expression levels in the six-event stack and the respective singles. The data indicate that there is no impact of the DvSnf7 dsRNA on the expression level. 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 × 1507 × MON 87411 × 59122 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins are similar in the six-event stack maize and in the single events. CP4 EPSPS and PAT shows the expected higher level in the stack resulting from the combination of events MON 87427 and MON 87411, and 1507 and 59122, respectively. Therefore, there is no indication of an interaction that may affect the integrity of the events and the levels of the newly expressed proteins in this stack. Based on the known biological function (Table 3) of the newly expressed proteins, the only foreseen interactions at the biological level are between the Cry proteins in susceptible insects, which will be dealt with in Sections 3.4.4.

3.4.2. Comparative analysis[10]

3.4.2.1. Overview of studies conducted for the comparative analysis

Application EFSA-GMO-NL-2017-139 presents data on agronomic and phenotypic characteristics, as well as on forage and grain composition of maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 (Table 5).

Table 5: Overview of the comparative analysis studies to characterise the six-event stack maize in application EFSA-GMO-NL-2017-139

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

GM: genetically modified.
(a): The field trials were located in Boone, IA; Jefferson, IA; Vermilion, IL; Warren, IL; Shelby, IL; Pawnee, KS; Miami, OH; and Berks, PA.
(b): MPA640B refers to LH244 × LH287.
(c): Non-GM reference varieties used in the 2014 field trials were LG2540, LG2548, Phillips 717, Channel 211-97, Midland Phillips 7B15P, Seed Consultants 1112, Stine 9724, Dekalb DKC62-06, Mycogen 2H721, NC+ 5220, NH6280, Stewart S602, Stewart S588, Channel 213-88, Dekalb DKC63-43, Mycogen 2J790, Gateway 6158, NH6769.

3.4.2.2. Experimental field trial design and statistical analysis

At each field trial site, the following materials were grown in a randomised complete block design with four replicates: the six-event stack maize exposed to the intended herbicides glyphosate and glufosinate-ammonium (treated), the six-event stack maize not exposed to the intended herbicides

[10] Dossier: Part II – Section 1.3 and additional information: 3/9/2018 and 29/8/2019.

(not treated), the comparator MPA640B and four non-GM maize reference varieties (hereafter referred to as 'non-GM reference varieties').

The agronomic, phenotypic and compositional data were analysed as specified by the EFSA GMO Panel (2010b, 2011a). This includes, for each of the two treatments of the six-event stack maize, the application of a difference test (between the GM maize and the non-GM comparator) and an equivalence test (between the GM maize and the set of non-GM commercial reference varieties). The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).[11]

3.4.2.3. Suitability of selected test materials

Selection of the test materials

To produce the GM stack maize, the single events MON 87427, MON 87460, MON 89034, 1507 and 59122 were transferred in the genetic background of the non-GM inbred lines LH287. Event MON 87411 was obtained transforming the non-GM inbred line LH244 and was maintained in this genetic background.

In subsequent subsections, maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 refers to hybrid (F_1) obtained crossing GM inbred line LH244 (carrying MON 87411) with GM inbred line LH287 (carrying MON 87427 × MON 87460 × MON 89034 ×1507 × 59122).

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 EFSA GMO Panel considers the selected comparator suitable for the comparative analysis. Maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 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.

Several 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 reference varieties were tested. On the basis of the provided information on relative maturity classes, the GMO Panel considers the selected non-GM reference varieties appropriate for the comparative assessment.

Seed production and quality

Seeds of maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 and the comparator used in the 2014 field trials were produced, harvested and stored under similar conditions. The seed lots were verified for their purity via event specific quantitative polymerase chain reaction analysis. The mean germination rates of maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 and of 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 six-event stack maize, the non-GM comparator and the non-GM reference varieties were properly selected and are of sufficient quality. Therefore, the test materials are considered acceptable 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 the US. The soil characteristics of the selected fields were diverse,[12] corresponding to optimal and near-optimal conditions for maize cultivation (Sys et al., 1993). The GMO Panel considers that the selected sites reflect commercial maize-growing regions in which the test materials are likely to be grown.

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 sites; therefore, the GMO Panel considers that the meteorological dataset falls within the range of climatic conditions normally occurring at these sites.

[11] 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).

[12] Soil types of the field trials were silty clay loam, loam and silty loam; soil organic matter ranged from 0.9% to 3.8%.

Management practices

The field trials included plots containing six-event stack maize, plots with the comparator and plots with non-GM reference varieties, managed according to local agricultural practices. In addition, the field trials included plots containing the six-event stack maize managed following the same agricultural practices, plus exposed to the glyphosate-containing and glufosinate-ammonium-containing herbicides. Glyphosate- and glufosinate-ammonium-containing herbicides were applied separately at the V2-V4 and V5-V7 growth stage, respectively. The GMO Panel considers that the management practices including sowing, harvesting and application of plant protection products were appropriate.

Conclusion on representativeness

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

3.4.2.5. Agronomic and phenotypic analysis

Thirteen[13] agronomic and phenotypic endpoints plus information on abiotic stressors, disease incidence and arthropod damage were collected from the field trials (Table 5). Of those, the endpoint dropped ears was not analysed as described in Section 3.4.2.2 because more than 90% of the values were 0.

The outcome of the analysis for the remaining 12 endpoints was as follows:

- For the six-event stack maize (not treated with the intended herbicides), statistically significant differences with the non-GM comparator were identified for early stand count, days to 50% pollen shed, days to 50% silking, root lodged plants, final stand count and test weight. All these endpoints fell under equivalence category I except for root lodged plants, for which the test of equivalence was not applied (because the variation between the non-GM commercial varieties was estimated to be 0).[14]
- For the six-event stack maize (treated with the intended herbicides), statistically significant differences with the non-GM comparator were identified for early stand count, days to 50% pollen shed, days to 50% silking, ear height, root lodged plants, final stand count and test weight. All these endpoints fell under equivalence category I or II except for root lodged plants, for which the test of equivalence was not applied.[14]

For the endpoint root lodged plants, significant differences were found between the six-event stack maize (for both treatments) and the non-GM comparator and equivalence could not be determined. Because the mean values for root lodged plants were very small for all the materials,[14] the GMO Panel considered that this result does not indicate issues in the materials used for the comparative analysis. Whether these differences could have an adverse environmental impact is discussed in Section 3.4.4.1.

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 (9 in forage and 69 in grain), including the key constituents recommended by the OECD (2002). A total 15 grain constituents were excluded from the statistical analysis since more than 50% of the observations were below the limit of quantification.[15]

The statistical analysis was applied to the remaining 63 constituents (9 in forage[16] and 54 in grain[17]); a summary of the outcome of the test of difference and the test of equivalence is presented in Table 6.

[13] 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 and yield.

[14] Estimated means for root lodged plants (number of plants/two rows): 1.61 (untreated GM maize), 0.67 (treated GM maize), 2.89 (non-GM comparator) and 0.25 (non-GM reference varieties).

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

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

[17] Proximates and fibre content (ash, carbohydrates, moisture, protein, total fat, ADF, NDF and total dietary fibre (TDF)), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium and zinc), vitamins (ß-carotene, thiamine, riboflavin, niacin, pyridoxine, folic acid and α-tocopherol), amino acids (alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine), fatty acids (palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C21:0) and behenic acid (C22:0)) and other compounds (ferulic acid, p-coumaric acid, phytic acid and raffinose).

- For the six-event stack maize not treated with the intended herbicides, statistically significant differences in the comparison with MPA640B were identified for 30 endpoints (3 in forage and 27 in grains). All these endpoints fell under equivalence category I or II except for acid detergent fibre (ADF) in forage, for which the test of equivalence was not applied (because the variation between the non-GM commercial varieties was estimated to be 0), and arginine levels in grain which fell under equivalence category III (Table 6).
- For the six-event stack maize treated with the intended herbicides, statistically significant differences in the comparison with MPA640B were identified for 39 endpoints (3 in forage and 36 in grains). All these endpoints fell under equivalence category I or II except for the levels of protein, arginine, glycine, leucine, lysine and manganese in grain, which fell under category III or IV (Table 6).

Table 6: Summary of the outcome of the comparative analysis in grain and forage from maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122

		Test of difference[a]			
		Not treated[c]		**Treated[c]**	
		Not different	**Significantly different**	**Not different**	**Significantly different**
Test of equivalence[b]	Category I/II	31	28[d]	21	33[d]
	Category III/IV	1[e]	1[f]	1[e]	6[f]
	Not categorised	1[g]	1[h]	2[g]	–
	Total endpoints	63		63	

(a): Comparison between the six-event stack maize and the non-GM comparator.
(b): Four different outcomes: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence). Not categorised means that the test of equivalence was not applied because of the lack of variation among the non-GM reference varieties.
(c): Treated/not treated with glyphosate and glufosinate-ammonium-containing herbicides.
(d): Endpoints with significant differences between the six-event stack maize and its non-GM comparator and falling in equivalence category I-II. For forage, not treated only: carbohydrates and protein. Treated only: moisture, calcium and phosphorus. Both treated and not treated: none. For grains, not treated only: protein, glycine, leucine and manganese. Treated only: ash, moisture, NDF, cystine, tryptophan, potassium, zinc and thiamine. Both treated and not treated: carbohydrates, total fat, TDF, alanine, glutamic acid, isoleucine, methionine, serine, threonine, valine, linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C20:1), calcium, copper, iron, magnesium, pyridoxine, ß-carotene, α-tocopherol, ferulic acid and raffinose.
(e): Proline levels in grain fell under equivalence category III (for both treated and not treated GM maize), but no significant differences were identified between the GM maize and the non-GM comparator.
(f): Endpoints with significant differences between the six-event stack maize and its non-GM comparator and falling in equivalence category III/IV. In forage, none. In grain, untreated only: none. Treated only: protein, glycine, leucine, lysine and manganese. Both treated and untreated: arginine. Quantitative results for these endpoints are reported in Table 7.
(g): Endpoints that were not categorised for equivalence and for which no significant differences were identified between the six-event stack maize and the non-GM comparator: ADF in forage (treated only) and NDF in forage (both treated and not treated).
(h): Level of ADF in forage (not treated only) was not categorised for equivalence and a significant difference was identified between the six-event stack maize and the non-GM comparator. Quantitative results are reported in Table 7.

The GMO Panel assessed all significant differences between the six-event stack maize and its non-GM comparator, taking into account the potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties. Quantitative results for the endpoints showing significant differences between the six-event stack maize and its non-GM comparator and not falling under equivalence category I/II are given in Table 7.

Table 7: Quantitative results (estimated means and equivalence limits) for compositional endpoints in maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 that are further assessed based on the results of the statistical analysis

	Endpoint	Maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122		Non-GM comparator	Non-GM reference varieties	
		Not treated[(a)]	Treated[(a)]		Mean	Equivalence limits
Forage	ADF (% dw)	25.78*	25.05	24.18	24.00	–
Grain	Protein (% dw)	10.78*	11.08*	10.21	9.88	8.82–10.94
	Arginine (% AA)	4.57*	4.45*	4.70	4.90	4.58–5.22
	Glycine (% AA)	3.56*	3.46 *	3.65	3.76	3.47–4.06
	Leucine (% AA)	13.39*	13.57*	13.10	12.90	12.33–13.48
	Lysine (% AA)	2.59	2.47*	2.64	2.83	2.58–3.08
	Manganese (mg/kg dw)	6.87*	7.38*	6.54	5.98	4.69–7.28

For maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122, significantly different values are marked with an asterisk, while the outcomes of the test of equivalence are differentiated by greyscale backgrounds. A white background is used for equivalence category I and II and for ADF in forage (which was not categorised for equivalence); light and dark grey backgrounds correspond to equivalence category III and IV, respectively.
dw: dry weight; %AA: percentage total amino acid.
(a): Treated/not treated with glyphosate- and glufosinate-ammonium-containing herbicides.

3.4.2.7. Conclusions on the comparative analysis

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

- None of the differences identified in the agronomic and phenotypic characteristics between the six-event stack maize and the non-GM comparator needs further assessment, except for the changes in root lodged plants. These differences are further assessed for their potential environmental impact in Section 3.4.4.
- None of the differences identified in forage and grain composition between the six-event stack maize and the non-GM comparator needs further food/feed safety assessment except for the changes in levels of ADF in forage and protein, arginine, glycine, leucine, lysine and manganese in grain. These differences are further discussed in Section 3.4.3.

3.4.3. Food/Feed safety assessment

3.4.3.1. Effects of processing

Maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 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 six-event stack maize into food and feed products is not expected to result in products being different from those of conventional non-GM maize varieties.

3.4.3.2. Influence of temperature and pH on newly expressed proteins

The effects of temperature and pH on proteins CP4 EPSPS, CspB, NPTII, Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1 and Cry35Ab1 newly expressed in this six-event stack maize have been previously evaluated by the GMO Panel (Table 1). No new information has been provided in the context of this application.

3.4.3.3. Toxicology

Testing of newly expressed proteins[18]

Ten proteins (CP4 EPSPS, CspB, NPTII, Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1 and Cry35Ab1) are newly expressed in the six–event stack maize (Section 3.4.1). The GMO Panel has previously assessed these proteins in the context of the single maize events and no safety concerns were identified for humans and animals. The GMO Panel is not aware of any other new information that would change its previous conclusions on the safety of these proteins.

The potential for a functional interaction between the proteins newly expressed in the six-event stack maize was assessed by the GMO Panel with regard to human and animal health. The CP4 EPSPS and PAT proteins are enzymes that catalyse distinct biochemical reactions and act on unrelated substrates in the plant with high substrate specificity. 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 aminoglycoside antibiotics (Fraley et al., 1983). The insecticidal proteins Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1 and Cry35Ab1 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 affinity to Cry proteins (Hammond et al., 2013; Koch et al., 2015).

On the basis of the known biological function of the individual newly expressed proteins (Table 3), there is currently no expectation for their possible interactions relevant to the food and feed safety of this six-event stack maize.

In vitro protein degradation studies on CP4 EPSPS, CspB, NPTII, Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins have been previously evaluated by the GMO Panel (Table 1) and no indications of safety concerns were identified.

The GMO Panel concludes that there are no safety concerns to human and animal health related to the proteins CP4 EPSPS, CspB, NPTII, Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1 and Cry35Ab1 newly expressed in maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122.

Testing of new constituent other than proteins[19]

No new constituents other than the newly expressed proteins have been identified in maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122, with the exception of the intended expression of DvSnf7 dsRNA and derived siRNAs, designed to control coleopteran pests via RNAi. The GMO Panel has previously assessed these compounds in the context of the single maize event MON 87411 (EFSA, 2018) and concluded that no safety concerns are associated with the presence of these compounds. The GMO Panel is not aware of any other new information that would change its previous conclusions on the safety of these compounds.

On the basis of the known biological function of these constituents (Table 3), there is currently no expectation for their possible interactions with other new compounds (newly expressed proteins) or other constituents relevant to the food and feed safety of this six–event stack maize.

Information on altered levels of food and feed constituent[20]

Acid detergent fibre in forage (not treated with the intended herbicides), and protein, glycine, leucine, lysine and manganese in grain (treated with the intended herbicides) and arginine in grain (under both herbicide regimens treatments) were significantly different in the six-stack maize when compared with its non-GM comparator and showed a lack of equivalence with the non-GM reference varieties (Section 3.4.2.6). No toxicological concern is identified regarding these compounds. Further information on safety is provided in Section 3.4.3.6.

[18] Dossier: PartII – Section 1.4.1.
[19] Dossier: PartII – Section 1.4.2.
[20] Dossier: PartII – Section 1.4.3.

Testing of the whole genetically modified food and feed[21]

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

In accordance to Regulation (EU) No 503/2013, the applicant provided 90-day oral repeated-dose toxicity studies in rats on whole food and feed from each of the maize single-events composing the six-event stack maize.

90-Day studies on maize MON 87427, MON 87460, MON 89034, and MON 87411

The GMO Panel had previously concluded that these studies are in line with Reg (EU) No 503/2013 and do not show adverse effects related to diets incorporating the single-event maize crops (MON 87427, MON 87460 and MON 89034 in EFSA GMO Panel (2019a); MON 87411 in EFSA GMO Panel (2018a,b,c).

90-Day study on maize 59122

A 90-day study on maize 59122 had been previously assessed by the GMO Panel in the context of the single-event application dossier (EFSA, 2007). Upon EFSA's request to fulfil the requirements of Regulation (EU) No 503/2013, the applicant confirmed that the test material was treated with the intended herbicide (glufosinate-ammonium-containing herbicide). Therefore, the GMO Panel concluded that this study is in line with the legal requirements and confirmed that do not show adverse effects related to a diet incorporating the single-event maize 59122. The incorporation rate of maize in this study is around 35%, 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.

90-Day study on maize 1507

The GMO Panel had previously assessed a 90-day study on maize 1507 in the context of the single-event application (EFSA, 2005a). Kernels used in that study were obtained from 1507 maize plants that had not been treated with the intended herbicide (glufosinate-ammonium-containing herbicide). Upon EFSA's request to fulfil the requirements of Regulation (EU) No 503/2013, the applicant provided a new 90-day study on 1507 maize. Pair-housed Crl:CD(SD) rats (16/sex per group; 2 rats/cage) were allocated to six groups using a randomised complete block design with 5 replications/sex. Groups were fed diets containing 50% by weight grains either from maize 1507 plants treated with the intended herbicide (test material, high dose), from the conventional counterpart (non-GM comparator, control material), or one of three non-transgenic commercial reference maize hybrids.[22] An additional group was fed diets containing 33% by weight grains from maize 1507 treated with the intended herbicide (test material, low dose) and 17% by weight maize grain from the conventional counterpart. The study was adapted from OECD test guideline 408 (2018), aligned with EFSA Scientific Committee guidance (2011) and complied with the principles of Good Laboratory Practice (GLP) with some deviations not impacting the study results and interpretation (i.e. test item stability, homogeneity and concentration), which are detailed below. Event-specific polymerase chain reaction (PCR) analysis confirmed the presence of the event 1507 in both the GM maize grains and diets and excluded the presence of the event in the respective controls. ELISA analyses also confirmed the presence of event 1507 (i.e. Cry1F concentration) in the GM maize grains and GM diets. Both GM and control maize grains and diets were analysed for nutrients, antinutrients and potential contaminants (e.g. selected heavy metals, mycotoxins and pesticides). Balanced diets were formulated based on the specifications for PMI Certified Rodent LabDiet® 5002. The stability of the test and control materials was not verified; however, in accordance to product expiration date declared by the diet manufacturer, the constituents of the diets are considered stable for the duration of the treatment. The GMO Panel considered this justification acceptable. Diet preparation procedures and regular

[21] Dossier: PartII – Section 1.4.4; additional information 10/12/2018; 17/1/2019; 14/3/2019; 27/2/2020 and 16/7/2020; spontaneous information 20/8/2019.

[22] Non-transgenic commercial reference maize hybrids: P0760, P0589 and XL5840.

evaluations of the mixing methods guaranteed the homogeneity and the proper concentration of the test or control substances in them. The applicant provided information on concentration of Cry1F protein in the formulated test diets, further supporting the homogeneity of the formulations. Feed and water were provided *ad libitum*. In-life procedures and observations and terminal procedures were conducted in accordance to OECD (2018).

In the statistical analysis, rats consuming the low- and high-dose test diets were compared with those consuming the control diet. For continuous parameters, a linear mixed model was applied to data from individual animals for the two sexes combined (fixed effects: diet, sex and sex-by-diet interaction; random effects: block-within-sex and cage). Test-control comparisons were done both across sexes and separately for males and females; in case a significant sex-by-diet interaction was identified, only the sex-specific results were considered for the assessment. The model was modified as needed for the analysis of sex-specific endpoints and cage-level data (food consumption and food efficiency).

There were no test diet-related incidents of mortality or clinical signs. All animals survived to scheduled euthanasia except one male from the reference group XL5840 that was found dead on test day 69 without any preceding clinical signs. Although a cause of death could not be conclusively determined, the incidental death of an untreated animal did not impact the interpretation of the study. No test diet-related adverse findings were identified in any of the investigated parameters. A small number of statistically significant findings were noted but these were not considered adverse effects of treatment for one or more of the following reasons:

- were present at the low dose but not in the high-dose groups;
- were within the normal variation for the parameter in rats of this age;
- were of small magnitude;
- were identified at only a small number of time intervals with no impact on the overall value;
- exhibited no consistent pattern with related parameters or endpoints.

Detailed description of statistically significant findings identified in rats given diets containing maize 1507 is reported in Appendix C.

No gross pathology findings related to the administration of the test diets were observed at necropsy, and the microscopic examinations of a wide range of organs and tissues did not identify relevant differences in the incidence and severity of the histopathological findings related to the administration of the test diet compared to the control group.

The GMO Panel concludes that this study is in line with the requirements of Regulation (EU) No 503/2013 and that no test diet related adverse effects were observed in rats after feeding diets including maize 1507 up to 50% for 90 days.

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 and adjuvanticity (Codex Alimentarius, 2009; EFSA GMO Panel, 2011a; Commission Regulation (EU) No 503/2013). Furthermore, an assessment of specific newly expressed proteins in relation to their potential to cause celiac disease was also performed (EFSA GMO Panel, 2017e).

Assessment of allergenicity of the newly expressed proteins[23]

For allergenicity, the GMO Panel has previously evaluated the safety of CP4 EPSPS, CspB, NPTII, Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins individually, and no evidence of allergenicity was identified in the context of the applications assessed (Table 1). No new information on allergenicity of the proteins newly expressed in this six-event stack maize that might change the previous conclusions of the GMO Panel has become available. Based on the current knowledge, and as there is no evidence of allergenicity of the newly expressed proteins, there are no expected concerns of allergenicity as a consequence of their interaction in this six–event stack maize.

The GMO Panel has previously evaluated the safety of the newly expressed proteins, and no evidence of adjuvanticity were identified in the context of the applications assessed (Table 2). More recently, this aspect has been discussed in detail by EFSA (EFSA, 2018; Parenti et al., 2019). To date, there is no evidence for adjuvanticity in the GMOs assessed by the Panel. This six–event stack maize

[23] Dossier: Part II – Sections 1.5.1 and 1.5.3.

has similar levels of the individual *Bt* proteins as those in the respective single maize events (see Section 3.4.1). The GMO Panel did not find indications that the *Bt* proteins at the levels expressed in this six-event stack maize might be adjuvants able to enhance an allergic reaction.

The applicant provided spontaneous information on the safety of the CP4 EPSPS, CspB, NPTII, Cry1A.105, Cry2Ab2 and Cry3Bb1 proteins regarding their potential to cause a celiac disease response.[24,25] For such assessment, the applicant followed the principles described in the EFSA GMO Panel guidance document (EFSA GMO Panel, 2017a–e). The assessment of these proteins identified no perfect or relevant partial matches with known celiac disease peptide sequences. These partial matches have been previously assessed by the EFSA GMO Panel (2019a,b,f, 2020), and no indications of safety concerns were identified.

Assessment of allergenicity of the whole GM plant[26]

The GMO Panel regularly reviews the available publications on food allergy to maize. However, maize is not considered a common allergenic food[27] (OECD, 2002). Therefore, the GMO Panel does not request experimental data to analyse the allergen repertoire of GM maize.

In the context of this application and considering the data from the molecular characterisation, the compositional analysis and the assessment of the newly expressed proteins (see Sections 3.4.1, 3.4.2 and 3.4.3), the GMO Panel identifies no indications of a potentially increased allergenicity of food and feed derived from this six-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, Cry1F, PAT, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins newly expressed in MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize. Dietary exposure was estimated based on protein expression levels reported in this application for the six-event stack maize treated with the intended herbicides, the current available consumption data and feed practices, the foods and feeds currently available in the market and the described processing conditions. Table 8 describes the protein expression levels derived from replicated field trials in United States during 2014 (five locations, four replicates) and used to estimate both human and animal dietary exposure to CP4 EPSPS, CspB, NPTII, Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins.

Table 8: 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 × 1507 × MON 87411 × 59122 maize treated with a combination of the intended herbicides[(a)]

Protein	Tissue/developmental stage	
	Grains/R6[(b)] (µg/g dry weight and µg/g fresh weight)	Forage/R5 (µg/g dry weight)
CP4 EPSPS[(c)]	12/10	200
CspB	0.11/0.099	0.13
NPTII	0.0058/0.0053[(d)]	0.24
Cry1A.105[(e)]	15/14	43
Cry2Ab2	2.4/2.2	40
Cry1F	2.6/2.3	7.3

[24] It is pointed out that the requirements laid down in the EFSA guidance on allergenicity (EFSA GMO Panel, 2017a–e) are not applicable to this dossier, as described in Section 1.5 'Transition period' of the above guidance document.
[25] Additional information: 18/6/2019 and 29/5/2020.
[26] Dossier: Part II – Section 1.5.2.
[27] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

Protein	Tissue/developmental stage	
	Grains/R6[(b)] (μg/g dry weight and μg/g fresh weight)	Forage/R5 (μg/g dry weight)
PAT[(c)]	0.025[(f)]	0.7
Cry3Bb1	5.1/4.6	46
Cry34Ab1	36/32	87
Cry35Ab1	1.1/1.0	21

(a): Intended herbicides: glyphosate and glufosinate–ammonium.
(b): Fresh weight values for CP4 EPSPS, CspB, NPTII, Cry1A.105, Cry2Ab2 and Cry3Bb proteins used to estimate human dietary exposure were calculated by multiplying the dry weight values by a dry weight correction factor (DWCF). Similarly, a DWCF was also used to convert the fresh weight values for Cry1F, PAT, Cry34Ab1, and Cry35Ab1 to dry weight values.
(c): CP4 EPSPS levels are the result of CP4 EPSPS protein expressed in MON 87427 and MON 87411. Likewise, PAT levels are the result of PAT protein expressed in maize 1507 and 59122.
(d): N = 15 since five samples were reported as below the LOQ (LOQ = 0.005 μg/g fw).
(e): Expression levels for Cry1A.105 are not corrected for the cross-reactivity between Cry1F and Cry1A.105 observed in the ELISA for Cry1A.105. As a consequence, the Cry1A.105 expression levels are overestimated by ~ 7%.
(f): PAT protein levels were below the limit of detection in all grain samples (LOD = 0.025 μg/kg). The reported LOD was used for both human and animal dietary exposure estimations.

Human and animal dietary exposure assessment to DvSnf7 dsRNA and its derived siRNAs was not conducted because these molecules are generally rapidly denaturated, depurinated and degraded shortly after ingestion, and therefore they are considered generally not to exert any biological effects once ingested by humans and animals (EFSA GMO Panel, 2018a).

Human dietary exposure[28]

Human dietary exposure was estimated across different European countries on different population groups[29]: young population (infants, toddlers, 'other children'), adolescents, adult population (adults, elderly and very elderly) and special populations (pregnant and lactating women).

Mean protein expression values on fresh weight basis (Table 8) are considered as the most adequate to estimate human dietary exposure (both acute and chronic) when working with raw primary commodities that are commonly consumed as processed blended commodities (EFSA, 2019). Since no specific consumption data were available on commodities containing, consisting of or obtained from the six-event stack 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] Corn oil was excluded from the assessment since no proteins are expected to be present in the oil.

For the acute dietary exposure estimations, the applicant directly assigned to processed commodities the mean value reported for the concentration of the newly expressed proteins in maize grains (Table 8). Overall, this is a conservative approach as neither recipes nor the effect of processing on the final concentration of newly expressed proteins are considered, except for corn oil which is eventually excluded from the exposure estimations. Summary statistics from the EFSA consumption database were used.[31] Acute dietary exposure in high consumers within each dietary survey and age class (toddlers, 'other children', adolescents, adults, elderly) 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). The highest acute dietary exposure was estimated in the age class 'Toddlers' with exposure estimates that ranged between 0.04 μg/kg body weight (bw) per day and 260 μg/kg bw per day for NPTII and Cry34Ab1 proteins, respectively. The most relevant food commodities in terms of contribution to the dietary exposure were sweet corn.

The GMO Panel estimated chronic dietary exposure to CP4 EPSPS, CspB, NPTII, Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins. Individual consumption data of the

[28] Dossier: Part II – Section 2.4 and Additional information 26/8/2019.
[29] For infants, very elderly population and vulnerable groups only chronic dietary exposure estimations are provided.
[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.

relevant food commodities were retrieved from the EFSA Consumption Database, using dietary surveys with at least two days consumption and covering a total of 23 European countries.[33] Different recipes and factors were considered to estimate the amount of maize in the consumed commodities before assigning newly expressed protein 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 percentile chronic exposure (highly exposed population) was derived from the distribution of the individual dietary exposure estimates within each dietary survey and age class. The highest chronic dietary exposure was estimated in the age class 'Infants' with exposure estimates that ranged between 0.024 μg/kg bw per day and 144 μg/kg bw per day for NPTII and Cry34Ab1 proteins, respectively. The main contributor to the exposure in the dietary survey with the highest estimates was sweet corn.

Consumption data on pollen supplements are available for few consumers across nine different European countries.[35] However, since no data on the presence of newly expressed proteins in pollen were available, the potential dietary exposure to CP4 EPSPS, CspB, NPTII, Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins from the consumption of pollen supplements could not be estimated.

Animal Dietary exposure[36]

Dietary exposure to CP4 EPSPS, CspB, NPTII, Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins newly expressed in maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 was estimated across different animal species, as below described, assuming the consumption of maize products commonly entering the feed supply chain (i.e. maize grains, gluten feed, gluten meal and forage). A conservative scenario with 100% replacement of conventional maize products by the six-event stack maize products was considered.

Mean levels (dry weight) of the newly expressed proteins in grains and forage from the six–event stack maize (treated with the intended herbicide) used for animal dietary exposure are listed in Table 8.

Mean levels (dry weight) of the newly expressed proteins in maize gluten feed and gluten meal were calculated to be, respectively, 2.6- and 7.1-fold than in grain, based on adjusting factors that take into account the protein content in these feed materials relative to maize grain (OECD, 2002), and assuming that no protein is lost during their production/processing.

The applicant estimated dietary exposure to CP4 EPSPS, CspB, NPTII, Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins via the consumption of maize grains, gluten feed and gluten meal in broiler, finishing pig and lactating dairy cow, based on estimates for animal body weight, daily feed intake and inclusion rates (percentage) of maize grains and forage in diets (OECD, 2009). Estimated dietary exposure in livestock animals is reported in Appendix D.

To further integrate the assessment, the GMO Panel estimated the dietary exposure to CP4 EPSPS, CspB, NPTII, Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins via the consumption of forage in beef cattle, dairy cattle, lamb, breeding pig and layer, based on estimates for animal body weight, daily feed intake and inclusion rates (percentage) of maize products in diets (OECD, 2009). Estimated dietary exposure in livestock animals is reported in Appendix D.

3.4.3.6. Nutritional assessment of endogenous constituents

The intended traits of maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 are tolerance to glyphosate- and glufosinate-ammonium-containing herbicides, insect resistance, and drought tolerance, with no intention to alter nutritional parameters. However, several compounds, ADF in forage, and crude protein, arginine, glycine, leucine, lysine and manganese in the six-event stack maize grain were significantly different from its conventional counterpart and showed a lack of equivalence with the set of non-GM reference varieties/could not be categorized (Section 3.4.2.6). The biological relevance of these compounds, the role of maize as contributor to

[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, Slovenia and Sweden.

[34] 100 grams of maize bread are made with approximately 74 g of maize flour, and a reverse yield factor of 1.22 from the conversion of maize grains into flour is used. This results in 26.2 μg of Cry34Ab1 per gram of maize bread as compared to 34 μg/g in the maize grains.

[35] https://www.efsa.europa.eu/en/food-consumption/comprehensive-database. Data accessed April 2020.

[36] Dossier: Part II – Section 2.4 and Additional Information 27/8/2019.

their total intake and the magnitude and direction of the observed changes were considered during the nutritional assessment.

Human nutritional assessment

The human nutritional assessment covered the observed changes in the levels of crude protein, arginine, glycine, leucine, lysine and manganese in grains (see Section 3.4.2.6)

A relatively small increase of protein content (5–8% as compared to its non-GM comparator) was observed in the GM-maize what does not imply any concern. Maize protein is considered of relatively low nutritional quality due to a poor balance of essential amino acids, in particular due to the low levels of lysine and tryptophan. Among the four amino acids under assessment, two of them are indispensable in humans: lysine and leucine. As compared to the non-GM comparator, the levels of leucine slightly increased (2–4%) while for lysine there was a decrease of ~ 6% (treated GM). Although the decrease in lysine implies that the amino acid composition of the GM maize might be less favourable, the higher amount of protein in the GM maize indicates a similar intake of this essential amino acid per amount of maize consumed.

Regarding manganese, an increase of 5–13% was observed as compared to the non-GM comparator. This mineral is an essential element for humans. Although high oral intake of manganese may be neurotoxic (SCF, 2000), no Tolerable Upper Intake Levels (UL) are set as a no observed adverse effect level (NOAEL) for critical endpoints from animal studies is not available and because of the limitations of the data in humans (EFSA NDA Panel, 2013). In addition, in maize, several minerals including manganese are usually bound to phytic acid what substantially decreases decreasing substantially their bioavailability (Gupta et al., 2015; Suri and Tanumihardjo, 2016).

Based on this assessment, it can be concluded that the compositional changes observed in maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 do not represent any concern from a nutritional point of view.

Animal nutritional assessment

The increase in ADF percentage in GM forage reported in Table 7 (Section 3.4.2.6) does not represent a nutritional concern for animals. Herbivorous animals consume feed materials with a high amount of fibre; therefore, the minimal difference observed is not considered relevant.

Maize grains are not considered a major source of proteins in animals; therefore, a small increase of protein content in grains (5–8% as compared to its non-GM comparator) rather improves the protein energy ratio.

Arginine and glycine are not considered essential amino acids, although Wu (2014) suggests that adequate supply of all amino acids is important to improve efficiency of animal production. The magnitude of the decrease of arginine and glycine observed in the GM maize as compared to the non-GM comparator does not represent a nutritional concern for animals.

Leucine and lysine are essential amino acids; the observed increase in leucine content (%AA) in maize grains is not a concern for animal nutrition. Also, the magnitude of the decrease of lysine (%AA) in maize grains does not pose a problem for animal nutrition. Both amino acids are usually balanced and supplemented in complete diets (e.g. for monogastric animals).

In animal nutrition, amino acids are also expressed as the percentage of dry weight (dw) to calculate directly the amount of each amino acid consumed daily by animals. According to Regulation (EC) No 767/2009[37], if, 'in complete or complementary feed, amino acids, vitamins and/or trace elements are indicated under the heading of analytical constituents, they shall be declared, along with the total amount thereof'. Therefore, variations in the amino acids described above were also assessed taking in consideration data expressed as % dw, which did not change the overall assessment. Less variability was reported compared to data expressed as % AA, for arginine, glycine and lysine, justified by the higher protein content of treated maize grain, and a slightly increase for leucine, similarly to the data expressed as % AA was detected.

Manganese is considered a trace element important for animal nutrition; the observed increase does not have any consequences, as complete diets are balanced with mineral premixes. Moreover, maize grains are a poor source of manganese (McDonald et al., 2011).

[37] Regulation (EC) No 767/2009 of the European Parliament and of the Council of 13 July 2009 on the placing on the market and use of feed, amending European Parliament and Council Regulation (EC) No 1831/2003 and repealing Council Directive 79/373/EEC, Commission Directive 80/511/EEC, Council Directives 82/471/EEC, 83/228/EEC, 93/74/EEC, 93/113/EC and 96/25/EC and Commission Decision 2004/217/EC. OJ L 229, 1.9.2009, p. 1–28.

3.4.3.7. Conclusion on the food/feed safety assessment

The newly expressed proteins CP4 EPSPS, CspB, NPTII, Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1 and Cry35Ab1 and the DvSnf7 dsRNA and derived siRNAs in maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 do not raise safety concerns for human and animal health. No interactions between the newly expressed proteins relevant for food and feed safety were identified, and no overall toxicological concerns on the six-event stack maize were identified. Similarly, the GMO Panel did not identify indications of safety concerns regarding allergenicity or adjuvanticity related to the presence of the newly expressed proteins in the six-stack maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122, or regarding the overall allergenicity of this six-event stack maize. Based on the outcome of the comparative assessment and the nutritional assessment, the GMO Panel concludes that the consumption of maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 does not represent any nutritional concern, in the context of the scope of this application.

3.4.4. Environmental risk assessment[38]

Considering the scope of application EFSA-GMO-NL-2017-139, which excludes cultivation, the environmental risk assessment (ERA) of maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 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 six–event stack maize grains during transportation and/or processing (EFSA GMO Panel, 2010a).

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, 2002). Field observations indicate that maize grains may survive and overwinter in some EU regions, resulting in volunteers in subsequent crops (e.g., Gruber et al., 2008; Palaudelmàs et al., 2009; Pascher, 2016). However, maize volunteers have been shown to grow weakly and flower asynchronously with the maize crop (Palaudelmàs et al., 2009). Thus, the establishment and survival of feral and volunteer maize in the EU is currently limited and transient.

It is unlikely that the intended traits of the six-event stack maize and the observed decrease in root lodged plants (see Section 3.4.2.5) will provide a selective advantage to maize plants, except when they are exposed to glyphosate- and/or glufosinate-ammonium-containing herbicides or infested by insect pests that are susceptible the DvSnf7 dsRNA and/or to the *Bt* proteins expressed by this GM maize. However, this fitness advantage will not allow the six-event stack maize to overcome other biological and abiotic factors (described above) limiting plant's persistence and invasiveness. Therefore, the presence of the intended traits and the observed differences in root lodged plants will not affect the persistence and invasiveness of the GM plant.

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

[38] Dossier: PartII – Section 4 and Additional Information 3/9/2018, 10/12/2018, 18/3/2020 and 29/5/2020.

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 an updated bioinformatic analysis for each of the single events in order to assess the possibility for HGT by homologous recombination.

The updated bioinformatic analyses for events MON 87427, MON 87460, MON 89034, 1507, MON 87411 and 59122 confirm the assessments provided in the context of previous applications (EFSA GMO Panel, 2019b,c,d,e,g)

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

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

Plant-to-plant gene transfer

The GMO Panel assessed the potential for occasional feral six-event stack maize plants originating from grain import spills to transfer recombinant DNA to sexually compatible plants; the environmental consequences of this transfer were also 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; Le Corre et al., 2020).

The potential of spilled maize grains to establish, grow and produce pollen is extremely low and transient (see Section 3.4.4.1). Therefore, likelihood/frequency of cross-pollination between occasional feral GM maize plants resulting from grain spillage, and weedy or cultivated *Zea* plants is considered extremely low (EFSA, 2016). Even if cross-pollination would occur, the GMO Panel is of the opinion that environmental effects as a consequence of the spread of genes from occasional feral GM maize plants in Europe will not differ from that of conventional maize varieties for the reasons given in Section 3.4.4.1, even if exposed to the intended herbicides.

3.4.4.3. Interactions of the GM plant with target organisms

Taking the scope of application EFSA-GMO-NL-2017-139 into account (no cultivation), potential interactions of occasional feral six-event stack maize plants arising from grain import spills with target organisms are not considered a relevant issue by the GMO Panel.

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 six-event stack maize grains is limited, and because ingested dsRNA and proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions of the six-event stack maize with non-target organisms are not considered by the GMO Panel to raise any environmental safety concern. Interactions that may occur between the Cry proteins (as mentioned in Section 3.4.1.4) would 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 six-event stack maize plants arising from grain import spills is limited, and because dsRNA and proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions with the abiotic environment and biogeochemical cycles are not considered by the GMO Panel to raise any environmental safety concern.

3.4.4.6. Conclusion of the environmental risk assessment

The GMO Panel concludes that it is unlikely that the six-event stack maize would differ from conventional maize varieties in its ability to persist under EU environmental conditions. Considering the scope of application EFSA-GMO-NL-2017-139, interactions of occasional feral six–event stack maize plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from the six-event stack maize to bacteria does not indicate a safety concern. Therefore, considering the combined traits, the outcome of the agronomic and phenotypic analysis, and the routes and levels of exposure, the GMO Panel concludes that the six-event stack maize would not raise safety concerns in the event of accidental release of viable GM maize grains into the environment.

3.4.5. Conclusion on the six-event stack maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122

No new data on the six single maize events MON 87427, MON 87460, MON 89034, 1507, MON 87411 and 59122 were identified that would lead to a modification of the original conclusions on their safety.

The combination of maize events MON 87427, MON 87460, MON 89034, 1507, MON 87411 and 59122 in the six-event stack maize did not give rise to issues concerning the molecular, agronomic, phenotypic or compositional characteristics that would be of concern for food and feed safety and nutrition.

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

Based on the biological functions of the newly expressed proteins, no indications of interactions between the events that would raise a safety issue were identified in MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122. Comparison of the levels of the newly expressed proteins between the six-event stack maize and those of the single maize events did not reveal an interaction at protein expression level. In addition, the potential impact of the DvSnf7 dsRNA on the levels of the newly expressed proteins was assessed by comparing the protein expression levels in the six-event stack and the respective single events. The data indicate that there is no impact of the DvSnf7 dsRNA on the expression levels of the newly expressed proteins.

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

No scientific information that could change the conclusions on this six–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 (January 2007–January 2020). The GMO Panel concludes that maize MON 87427 × MON 87460 × MON 89034 ×1507 × MON 87411 × 59122, as described in this application, does not raise any nutritional concern and is as safe as the comparator and the selected non-GM reference varieties.

3.5. Risk assessment of the subcombinations

Subcombinations previously assessed in the frame of other applications are discussed in Section 3.5.1. The subcombinations that have not been previously assessed are discussed in Section 3.5.2.

3.5.1. Subcombinations previously assessed

The GMO Panel has previously assessed 17 subcombinations (see Table 2) and no safety concerns were identified. Literature searches covering the 10 years before submission of the application and the period since the time of validity of the application (January 2007–January 2020) 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.

[39] Dossier: Part II – Section 7; additional information: 18/3/2020.

3.5.2. Subcombinations not previously assessed

Thirty-nine of the 56 subcombinations included in the scope of this application have not been previously assessed by the GMO Panel, and no experimental data were provided for these maize stacks (see Table 9). In this case, following the strategy defined by the GMO Panel,[40] the risk assessment takes as its starting point the assessment of the single maize events, and uses the data generated for the six-event stack as well as all the additional data available on subcombinations previously assessed by the GMO Panel (Table 2).

Table 9: Maize stacks not previously assessed and covered by the scope of application EFSA-GMO-NL-2017-139

Degree of stacking	Event
Five-event stack	59122 × MON 89034 × MON 87460 × MON 87427 × MON 87411
	1507 × MON 89034 × MON 87460 × MON 87427 × MON 87411
	1507 × 59122 × MON 87460 × MON 87427 × MON 87411
	1507 × 59122 × MON 89034 × MON 87427 × MON 87411
	1507 × 59122 × MON 89034 × MON 87460 × MON 87411
	1507 × 59122 × MON 89034 × MON 87460 × MON 87427
Four-event stack	MON 89034 × MON 87460 × MON 87427 × MON 87411
	59122 × MON 87460 × MON 87427 × MON 87411
	59122 × MON 89034 × MON 87427 × MON 87411
	59122 × MON 89034 × MON 87460 × MON 87411
	59122 × MON 89034 × MON 87460 × MON 87427
	1507 × MON 87460 × MON 87427 × MON 87411
	1507 × MON 89034 × MON 87427 × MON 87411
	1507 × MON 89034 × MON 87460 × MON 87411
	1507 × MON 89034 × MON 87460 × MON 87427
	1507 × 59122 × MON 87427 × MON 87411
	1507 × 59122 × MON 87460 × MON 87411
	1507 × 59122 × MON 87460 × MON 87427
	1507 × 59122 × MON 89034 × MON 87411
	1507 × 59122 × MON 89034 × MON 87460
Three-event stack	MON 87460 × MON 87427 × MON 87411
	MON 89034 × MON 87460 × MON 87411
	59122 × MON 87427 × MON 87411
	59122 × MON 87460 × MON 87411
	59122 × MON 87460 × MON 87427
	59122 × MON 89034 × MON 87411
	59122 × MON 89034 × MON 87460
	1507 × MON 87427 × MON 87411
	1507 × MON 87460 × MON 87411
	1507 × MON 87460 × MON 87427
	1507 × MON 89034 × MON 87411
	1507 × MON 89034 × MON 87460
	1507 × 59122 × MON 87411
	1507 × 59122 × MON 87460
Two-event stack	MON 87460 × MON 87411
	59122 × MON 87411
	59122 × MON 87460
	1507 × MON 87411
	1507 × MON 87460

[40] 115th GMO Panel meeting (Annex 1 of the minutes: http://www.efsa.europa.eu/sites/default/files/event/170517-m.pdf).

3.5.2.1. Stability of the events

The genetic stability of the inserted DNA over multiple generations in the six single maize events was demonstrated previously (see Table 2). Integrity of the events was demonstrated in the six-event stack maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 (Section 3.4.1.2) and the previously assessed maize subcombinations (see Table 2). The GMO Panel finds no reasons to expect the loss of integrity of the events in the maize subcombinations not previously assessed (see Table 9).

3.5.2.2. Expression of the events

The GMO Panel assessed whether any combination of the six 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 39 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 six-event stack maize. The levels were similar in the six-event stack maize and in the single events except for CP4 EPSPS and PAT, which showed, in general, the expected higher level in the stack resulting from the combination of the single events MON 87427 and MON 87411 for the CP4 EPSPS and the combination of 1507 and 59122 for the PAT (Section 3.4.1.3 and Appendix B). In addition, the potential impact of the DvSnf7 dsRNA on the levels of the newly expressed proteins was assessed by comparing the protein expression levels in the six-event stack and the respective singles. The data indicate that there is no impact of the DvSnf7 dsRNA on the expression levels of the newly expressed proteins. This supports the conclusion that interactions affecting the expression levels of the newly expressed proteins are not expected in the 39 subcombinations not previously assessed and included in the scope of application EFSA-GMO-NL-2017-139.

3.5.2.3. Potential functional interactions between the events

The GMO Panel assessed the potential for interactions between maize events in the 39 subcombinations not previously assessed (Table 9), taking into consideration intended traits and unintended effects.

Based on the known biological functions of the individual newly expressed proteins and dsRNA (Table 4), there is currently no expectation for possible interactions relevant for the food and feed or environmental safety between these proteins in those subcombinations. The GMO Panel took into account all the intended and potential unintended effects considered in the assessment of the six single events, the previously assessed subcombinations (Table 2) and the six-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 six-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 39 subcombinations included in the scope of application EFSA-GMO-NL-2017-139, for which no experimental data have been provided, the GMO Panel assessed the possibility of interactions between the events and concluded that these combinations would not raise safety concerns. These subcombinations are therefore expected to be as safe as the single maize events, the previously assessed subcombinations and the six-event stack maize.

3.6. Post-market monitoring

3.6.1. Post-market monitoring of GM food/feed

The GMO Panel concluded that the six-event stack maize, as described in this application, does not raise any nutritional concern and is as safe as the non-GM comparator and the selected non-GM reference varieties (Section 3.4.3.7). Seventeen of the subcombinations have been previously assessed and no safety concerns were identified. The 39 subcombinations not previously assessed and included in the scope of this application are expected to be as safe as the single maize events, the previously

assessed maize subcombinations and the six–event stack maize (Section 3.5.3). Therefore, the GMO Panel considers that post-market monitoring of food and feed from the six-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.

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 does not identify potential adverse environmental effects from the six-event stack maize, no case-specific monitoring is required.

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

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

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

3.6.3. Conclusion on post-market monitoring

No PMM 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 × 1507 × MON 87411 × 59122.

4. Overall conclusions

The GMO Panel was asked to carry out a scientific assessment of maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 and subcombinations for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003.

No new information was identified on the six single maize events (MON 87427, MON 87460, MON 89034, 1507, MON 87411 and 59122) that would lead to a modification of the original conclusions on their safety.

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

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

Since no new data were identified on the 17 previously assessed subcombinations that would lead to a modification of the original conclusions on their safety, the GMO Panel considers that its previous conclusions on these maize stacks remain valid. For the remaining 39 subcombinations included in the scope of application EFSA-GMO-NL-2017-139, no information has been provided. The GMO Panel assessed the possible interactions between the events in the 39 subcombinations and concludes that these combinations of events MON 87427, MON 87460, MON 89034, 1507, MON 87411 and

59122 would not raise safety concerns. These subcombinations are therefore expected to be as safe as the maize single events, the previously assessed subcombinations and the six-event stack maize.

The literature searches did not identify any relevant publications on maize MON 87427, MON 87460, MON 89034, 1507, MON 87411 and 59122. 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 A. This new information does not raise any concern for human and animal health and the environment regarding the six-event stack maize and its subcombinations.

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

In conclusion, the GMO Panel considers that maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 and its subcombinations, as described in this application, are as safe as the non-GM comparator and the selected non-GM reference varieties with respect to potential effects on human and animal health and the environment.

5. Documentation as provided to EFSA

1) Letter from the Competent Authority of Netherlands received on 21 February 2017 concerning a request for authorisation of the placing on the market of maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 (EFSA-GMO-NL-2017-139) submitted in accordance with Regulation (EC) No 1829/2003 by Monsanto Company.
2) Application EFSA-GMO-NL-2017-139 validated by EFSA, 31 May 2017.
3) Request for supplementary information to the applicant, 1 June 2018.
4) Request for supplementary information to the applicant, 6 July 2018.
5) Request for supplementary information to the applicant, 17 July 2018.
6) Receipt of supplementary information from the applicant, 3 September 2018.
7) Receipt of supplementary information from the applicant, 17 September 2018.
8) Request for supplementary information to the applicant, 16 October 2018.
9) Request for supplementary information to the applicant, 13 November 2018.
10) Receipt of supplementary information from the applicant, 10 December 2018.
11) Receipt of supplementary information from the applicant, 19 January 2019.
12) Receipt of supplementary information from the applicant, 14 March 2019.
13) Request for supplementary information to the applicant, 18 March 2019.
14) Request for supplementary information to the applicant, 16 April 2019.
15) Request for supplementary information to the applicant, 21 May 2019.
16) Receipt of supplementary information from the applicant, 19 June 2019.
17) Supplementary information submitted spontaneously by the applicant, 20 August 2019.
18) Receipt of supplementary information from the applicant, 27 August 2019.
19) Supplementary information submitted spontaneously by the applicant, 29 August 2019.
20) Receipt of supplementary information from the applicant, 30 August 2019.
21) Request for supplementary information to the applicant, 15 October 2019.
22) Receipt of supplementary information from the applicant, 17 December 2019.
23) Receipt of supplementary information from the applicant, 27 February 2020.
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Abbreviations

AA	amino acids
ADF	acid detergent fibre

BUN	blood urea nitrogen
bw	body weight
CaMV	cauliflower mosaic virus
CspB	cold shock protein B
CRM	comparative relative maturity
CTP	chloroplast transit peptide
dsRNA	double strand RNA
dw	dry weight
ELISA	enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphat 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
hsp	heat shock proteins
LOQ	limit of quantification
MS	Member States
NDF	neutral detergent fibre
NOAEL	no-observed-adverse-effect level
nos	nopaline synthase
NPTII	neomycin phosphotransferase II
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PAT	phosphinothricin-acetyl-transferase
PCR	polymerase chain reaction
PMEM	post-market environmental monitoring
RNA	ribonucleic acid
RNAi	RNA interference
siRNA	small interfering RNA
TDF	total dietary fibre
TSH	thyroid hormones
UL	tolerable upper intake level
UTR	untranslated region
WCR	western corn rootworm

Appendix A – Additional studies

List of additional studies performed by or on behalf of the applicant with regard to the evaluation of the safety of maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 for humans, animal or the environment

Study identification	Title
Hoi and Asiimwe (2015)	Phenotypic Evaluation and Environmental Interactions of Maize MON 87427 × MON 87460 × MON 89034 × TC1507 × MON 87411 × DAS-59122-7 in 2014 U.S. Field Trials.
Hoi (2015)	Phenotypic Evaluation of Maize MON 87427 × MON 87460 × MON 89034 × TC1507 × MON 87411 × DAS-59122-7 with Herbicide Treatments in 2014 U.S. Field Trials.
Joshi et al. (2016)	Comparison of Lipid Transfer Protein (LTP) Expression Levels from MON 87427 × MON 87460 × MON 89034 × TC1507 × MON 87411 × DAS–59122–7 with Conventional Control Maize.
Klusmeyer et al. (2016a)	Compositional Analyses of Maize Forage and Grain from MON 87427 × MON 87460 × MON 89034 × TC1507 × MON 87411 × DAS-59122-7 Grown in the United States in 2014.
Klusmeyer et al. (2016b)	Compositional Analyses of Maize Forage and Grain from MON 87427 × MON 87460 × MON 89034 × TC1507 × MON 87411 × DAS–59122–7 Grown in the United States in 2014: Individual Site Analysis.
Mueller and Uffman (2016)	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 × TC1507 × MON 87411 × DAS–59122–7 with the European Corn Borer (*Ostrinia nubilalis*).
Skottke and Malven (2015a)	Amended Report for MSL0026334: Southern Blot Analyses to Confirm the Presence of TC1507 and DAS–59122–7 in the Combined Trait Maize Product MON 87427 × MON 87460 × MON 89034 × TC1507 × MON 87411 × DAS–59122–7.
Skottke and Malven (2015b)	Southern Blot Analyses to Confirm the Presence of MON 87427, MON 87460, MON 89034 and MON 87411 in the Combined Trait Maize Product MON 87427 × MON 87460 × MON 89034 × TC1507 × MON 87411 × DAS–59122–7.
MSL0027263	An Acute Oral Gavage Toxicity Study of E. coli-produced Cry3Bb1 protein in CD–1 Mice

Appendix B – Protein expression data

Mean, standard deviation and range of protein levels (μg/g dry weight) from maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 (treated with glyphosate and glufosinate ammonium), MON 87427 (treated with glyphosate), MON 87460, MON 89034, 1507 (treated with glufosinate), MON 87411 (treated with glyphosate) and 59122 (treated with glufosinate ammonium) from field trials performed across five locations in the USA, in 2014 (n = 20, unless otherwise stated).

Protein	Event(s)	Leaf (V3-V4)	Root (V3-V4)	Forage (R5)	Grain (R6)
CP4 EPSPS	**MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122**	860[a] ± 100[b] (680–1,100)[c]	320 ± 61 (220–440)	200 ± 52 (130–280)	12 ± 2.0 (8.4–17)
	MON 87427	870 ± 100 (670–1,000)	290 ± 56 (200–390)	180 ± 68 (60–280)	9.3 ± 1.6 (6.7–12)
	MON 87411	54 ± 20 (40–130)	65 ± 15[d] (41–95)	12 ± 3.7 (7.7–20)	4.7 ± 1.1 (2.6–6.5)
CspB	**MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122**	2.1 ± 0.54 (1.4–3.1)	1.7 ± 0.60 (0.84–3.1)	0.13 ± 0.032 (0.093–0.20)	0.11 ± 0.014 (0.087–0.14)
	MON 87460	2.4 ± 0.60 (1.2–3.2)	2.0 ± 0.73 (0.47–2.9)	0.12 ± 0.027[d] (0.083–0.19)	0.099 ± 0.022 (0.073–0.14)
NPTII	**MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122**	5.4 ± 0.94 (3.7–7.0)	1.1 ± 0.16 (0.69–1.3)	0.24 ± 0.058 (0.14–0.36)	0.0058 ± 0.00035[d] (0.0055–0.0064)
	MON 87460	5.1 ± 1.3 (3.6–7.8)	1.1 ± 0.26 (0.67–1.8)	0.22 ± 0.053[d] (0.091–0.33)	0.0060 ± 0.00045[d] (0.0056–0.0065)
Cry1A.105	**MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122**	730 ± 370 (250–1,200)	80 ± 19 (52–110)	39 ± 13 (6.6–64)	14 ± 4.6 (6.3–20)
	MON 89034	880 ± 220 (270–1,300)	79 ± 17 (56–120)	26, ± 6.6[d] (16–37)	7.2 ± 3.7[d] (4.1–16)
Cry2Ab2	**MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122**	170 ± 39 (48–230)	160 ± 60 (31–270)	40 ± 11 (14–59)	2.4 ± 0.42 (1.6–3.4)
	MON 89034	160 ± 27 (120–200)	170 ± 47 (79–270)	30 ± 6.3[d] (21–41)	2.6 ± 0.54[d] (1.8–3.8)
Cry1F	**MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122**	28 ± 6.2 (19–38)	12 ± 2.0 (8.8–16)	7.3 ± 2.0 (4.0–11)	2.6 ± 0.35 (1.9–3.4)
	1507	27 ± 6.2 (18–39)	11 ± 1.9 (6.5–13)	6.8 ± 2.3 (3.5–12)	2.4 ± 0.32 (1.7–3.0)
PAT	**MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122**	16 ± 3.9 (9.1–23)	1.6 ± 0.49 (0.77–2.4)	0.70 ± 0.34 (0.18–1.4)	< LOD
	1507	4.2 ± 0.66 (3.2–5.2)	0.49 ± 0.17 (0.20–0.86)	0.45 ± 0.31[d] (0.16–1.0)	< LOD
	59122	11 ± 3.7 (4.6–16)	1.3 ± 0.29 (0.81–1.8)	0.59 ± 0.26[d] (0.14–0.98)	< LOD

Protein	Event(s)	Leaf (V3-V4)	Root (V3-V4)	Forage (R5)	Grain (R6)
Cry3Bb1	**MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122**	330 ± 56 (180–450)	320 ± 69 (170–420)	46 ± 13 (28–79)	5.1 ± 0.93 (3.7–6.8)
	MON 87411	320 ± 67 (190–510)	320 ± 89[d] (140–450)	42 ± 12 (20–72)	5.2 ± 0.91 (4.1–7.4)
Cry34Ab1	**MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122**	51 ± 11 (35–73)	64 ± 14 (47–92)	87 ± 32 (37–140)	36 ± 7.2 (25–52)
	59122	48 ± 13 (32–67)	60 ± 15 (45–98)	77 ± 29 (26–140)	37 ± 7.4 (25–51)
Cry35Ab1	**MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122**	39 ± 12 (21–62)	41 ± 8.3 (27–59)	21 ± 9.7 (6.5–37)	1.1 ± 0.29 (0.67–1.6)
	59122	38 ± 13 (21–60)	36 ± 7.7 (23–53)	22 ± 9.7 (4.1–34)	1.2 ± 0.31 (0.48–1.7)

LOD: limit of detection.
(a): Mean.
(b): Standard deviation.
(c): Range.
(d): n: Number of samples is 20 except for: n = 19 for CP4 EPSPS in root of MON 87411, for CspB in forage of MON 87460, for NPTII in forage for MON 87460, for Cry1A.105 in grain of MON 89034, for Cry2Ab2 in grain of MON 89034, for PAT in forage of 59122, for Cry3Bb1 in root of MON 87411; n = 18 for Cry1A.105 in forage of MON 89034, for Cry2Ab2 in forage of MON 89034, n = 13 for PAT in forage of 1507; n = 5 for NPTII in grain of MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122; n = 3 for NPTII in grain of MON 87460.

Appendix C – Statistically significant findings in the 90-day toxicity study in rats on the whole food/feed from maize 1507

Statistically significant parameter/endpoint	Finding	GMO Panel interpretation
Mean body weight gain	Increased	Not of toxicological relevance – sporadic, certain time points only – no effect on overall body weight or body weight gain
Forelimb grip strength	Reduced in high dose animals	Not of toxicological relevance – small magnitude (< 20% males, < 10% females) – within normal variability for this parameter
Motor activity	Reduced in low dose females increased in low dose males	Not of toxicological relevance – within the normal variability for this parameter – no consistent pattern – no significant change in high dose animals
Red blood cell and white blood cell parameters	Changes in both dose groups	Not of toxicological relevance – of low magnitude – no consistent pattern of findings within a group or between dose groups
Thyroid hormones (Thyroid Stimulating Hormone-TSH; Thyroxin -T4)	Increases in high dose females	Not of toxicological relevance – of low magnitude (12%) – no pathological changes in thyroid glands
Urine volume and specific gravity	Increased urine volume and decreased specific gravity in females	Not of toxicological relevance – values are within the physiological range – no associated changes in blood urea nitrogen (BUN) or kidney pathology

Appendix D – Animal dietary exposure

Table D.1: Dietary exposure to CP4 EPSPS, CspB, NPTII, Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1, and Cry35Ab1 proteins (μg/kg bw per day) in livestock based on the consumption of maize grains, gluten feed and gluten meal

	Dietary exposure (μg/kg bw per day)		
	Broiler	**Finishing pig**	**Lactating dairy cow**
CP4 EPSPS	1,415	695	1,154
CspB	13	6	11
NPTII	0.7	0.3	0.6
Cry1A.105	1,768	869	1,442
Cry2Ab2	283	139	231
Cry1F	306	151	250
PAT	2.9	1.4	2.4
Cry3Bb1	601	295	490
Cry34Ab1	4,244	2,084	3,462
Cry35Ab1	130	64	106

Table D.2: Dietary exposure to CP4 EPSPS, CspB, NPTII, Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1, and Cry35Ab1 proteins (μg/kg bw per day) in livestock based on the consumption of maize forage

	Dietary exposure (μg/kg bw per day)				
	Beef cattle	**Dairy cattle**	**Lamb**	**Breeding pig**	**Layer**
CP4 EPSPS	3,840	4,615	2,550	923	1,368
CspB	2.5	3	1.6	0.6	0.9
NPTII	4.6	5.5	3.1	1.1	1.6
Cry1A.105	826	992	548	198	294
Cry2Ab2	768	923	510	185	274
Cry1F	140	168	93.1	33.7	49.9
PAT	13.4	16.1	8.9	3.2	4.8
Cry3Bb1	883	1,061	586	212	315
Cry34Ab1	1,670	2,007	1,109	401	595
Cry35Ab1	403	485	268	96.9	144