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Assessment of genetically modified maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 for food and feed uses, under regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2018-151)

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Abstract

Genetically modified maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 was developed by crossing to combine five single events: MON 89034, 1507, MIR162, NK603 and DAS-40278-9. The GMO Panel previously assessed the five single maize events and 16 of the subcombinations and did not identify safety concerns. No new data on the single maize events or the assessed subcombinations were identified that could lead to the 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 in the five-event stack maize does not give rise to food and feed safety and nutritional concerns. The GMO Panel concludes that five-event stack maize, as described in this application, is as safe as the non-GM comparator and non-GM maize varieties tested. In the case of accidental release of viable five-event stack maize grains into the environment, this would not raise environmental safety concerns. The GMO Panel assessed the likelihood of interactions among the single events in nine of the 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 five-event stack maize. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9. Post-market monitoring of food/feed is not considered necessary. The GMO Panel concludes that the five-event stack maize and its subcombinations are as safe as its non-GM comparator and the tested non-GM maize varieties with respect to potential effects on human and animal health and the environment.

Keywords: genetic engineering, herbicide tolerant, insect resistant, maize (*Zea mays*), import and processing

Requestor: Competent Authority of The Netherlands

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Summary

Following the submission of application EFSA-GMO-NL-2018-151 under Regulation (EC) No 1829/2003 from Dow AgroSciences LLC as represented by Dow AgroSciences Belgium B.V. (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) herbicide-tolerant and insect-resistant maize (*Zea mays* L.) MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 (referred to hereafter as 'five-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-2018-151 is for import, processing and food and feed uses within the European Union (EU) of maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9, and does not include cultivation in the EU. The term 'subcombination' refers to any combination of up to four of the events present in the five-event stack maize. The safety of subcombinations that have either been or could be produced by crossing through targeted breeding approaches, and which can be bred, produced and marketed independently of the five-event stack, are risk assessed separately in the present scientific opinion.

The five-event stack maize was produced by crossing to combine five single maize events: MON 89034 expressing Cry1A.105 and Cry2Ab2 (for protection against certain lepidopteran pests), 1507 expressing Cry1F (for protection against certain lepidopteran pests) and PAT protein (for tolerance to glufosinate-ammonium-containing herbicides), MIR162 expressing Vip3Aa20 (for protection against certain lepidopteran pests) and PMI (selectable marker), NK603 expressing CP4 EPSPS (for tolerance to glyphosate-containing herbicides) and DAS-40278-9 expressing AAD-1 (to confer tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D) and the aryloxyphenoxypropionate (AOPP) containing herbicides).

The GMO Panel evaluated the five-event stack maize and its subcombinations with reference to the scope and appropriate principles described in its 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-2018-151, 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-2018-151, previous assessments of the five single events (MON 89034, 1507, MIR162, NK603 and DAS-40278-9), and 16 of the subcombinations provided a basis for the assessment of the five-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 five single maize events was identified by the updated bioinformatic analyses, nor reported by the applicant since the publication of the previous GMO Panel scientific opinions. Therefore, the GMO Panel considers that its previous conclusions on the safety of the single maize events remain valid.

For the five-event stack maize, the risk assessment included the molecular characterisation of the inserted DNA and analysis of protein expression. An evaluation of the comparative analysis of agronomic, phenotypic and compositional characteristics was carried out, and the safety of the newly expressed proteins and the whole food and feed were evaluated with respect to potential toxicity, allergenicity and nutritional characteristics. Environmental impacts and post-market environmental monitoring (PMEM) plan were also evaluated. The molecular characterisation data establish that the events MON 89034, 1507, MIR162, NK603 and DAS-40278-9 combined in the five-event stack maize have retained their integrity. Protein expression analysis showed that the levels of the newly expressed proteins are similar in the five-event stack maize and in the single events.

Considering the selection of test materials, the field trial sites and the associated management practices and the agronomic–phenotypic characterisation as an indicator of the overall field trial quality, the GMO Panel concludes that the field trials were appropriate to support the comparative analysis. The comparative analysis of agronomic and phenotypic characteristics and grain and forage composition identified no differences between maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 and the non-GM comparator (referred to hereafter as comparator) that required further assessment except for the changes for the levels in grain of: ash, behenic acid (C22:0), arginine, glycine, histidine, phosphorus, potassium, phytic acid, lysine and pyridoxine. These changes were further assessed for food/feed safety 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 in the five-event stack maize does not give rise to food and feed safety and nutritional concerns. The GMO Panel concludes that maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 is as safe as the

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 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 would not raise safety concerns in the case of accidental release of viable GM maize grains into the environment.

Since no new safety concerns were identified for the 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 subcombinations included in the scope of application EFSA-GMO-NL-2018-151, no experimental data were provided. The GMO Panel assessed the possibility of interactions between the events in these 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 five-event stack maize.

Given the absence of safety concerns for foods and feeds from maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 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 five-event stack maize and its subcombinations. Based on the relevant publications identified through the literature searches, the GMO Panel does not identify any safety issue pertaining to the intended uses of maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 and its subcombinations.

The GMO Panel concludes that maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 and its subcombinations, as described in this application, are as safe as the 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 the application EFSA-GMO-NL-2018-151 is for food and feed uses, import and processing of the genetically modified (GM) of the herbicide-tolerant and insect-resistant maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 and all its subcombinations independently of their origin and does not include cultivation in the European Union (EU).

1.1. Background

On 31 May 2018, the European Food Safety Authority (EFSA) received from the Competent Authority of The Netherlands application EFSA-GMO-NL-2018-151 for authorisation of maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 (Unique Identifier MON-89Ø34-3 × DAS-Ø15Ø7–1 × SYN-IR162-4 × MONØØ6Ø3–6 × DAS-4Ø278-9 and its subcombinations), submitted by Dow AgroSciences LLC according to Regulation (EC) No 1829/2003[1]. Following receipt of application EFSA-GMO-NL-2018-151, EFSA informed EU Member States (MS) and the European Commission, and made the application available to them. Simultaneously, EFSA published a summary of the application.[2]

EFSA checked the application for compliance with the relevant requirements of Regulation (EC) No 1829/2003 and Regulation (EU) No 503/2013[3], with the EFSA guidance documents, and, when needed, asked the applicant to supplement the initial application. On 15 October 2018, EFSA declared the application valid.

From validity date, EFSA and the panel on genetically modified organisms of the European Food Safety Authority (referred to hereafter as 'GMO Panel') endeavoured to respect a time limit of 6 months to issue a scientific opinion on application EFSA-GMO-NL-2018-151. Such time limit was extended whenever EFSA and/or GMO Panel requested supplementary information to the applicant. According to Regulation (EC) No 1829/2003, any supplementary information provided by the applicant during the risk assessment was made available to the EU Member States and European Commission (for further details, see the section 'Documentation', below). In accordance with Regulation (EC) No 1829/2003, EFSA consulted the nominated risk assessment bodies of EU Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC[4]. The EU Member States had 3 months to make their opinion known on application EFSA-GMO-NL-2018-151 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 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 in the context of its scope as defined in application EFSA-GMO-NL-2018-151.

According to Regulation (EC) No 1829/2003, this scientific opinion is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation and thus will be part of the EFSA overall opinion in accordance with Articles 6(5) and 18(5). In addition to the present scientific opinion, EFSA was also asked to report on the particulars listed under Articles 6(5) and 18(5) of Regulation (EC) No 1829/2003, but not to give an opinion on them because they pertain to risk management.[2]

2. Data and methodologies

2.1. Data

The GMO Panel based its scientific assessment of five-event stack maize on the valid application EFSA-GMO-NL-2018-151, 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

[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: https://open.efsa.europa.eu/study-inventory/EFSA-Q-2018-00457
[3] Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. OJ L157, 8.6.2013, p. 1–48.
[4] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/NLC. OJ L 106, 12.3.2001, p. 1–38.

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 1829/2003, the applicable guidelines (i.e. EFSA GMO Panel, 2010a, 2011a,b, 2015, 2017a; EFSA Scientific Committee, 2011) and explanatory notes and statements (i.e. EFSA GMO Panel, 2010b, 2021e; EFSA, 2010, 2014, 2017, 2019a,b) for the risk assessment of GM plants.

For this application, in the context of the contract OC/EFSA/GMO/2018/02, the contractor performed preparatory work for the evaluation of the methods applied for the statistical analysis on maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9.

3. Assessment

3.1. Introduction

Application EFSA-GMO-NL-2018-151 covers the five-event stack maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 and all its 25 subcombinations independently of their origin (Table 1).

Table 1: The 26 combinations of the events covered by the scope of application EFSA-GMO-NL-2018-151

Degree of stacking	Event	Unique identifiers
5- event stack	MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9	MON-89Ø34-3 × DAS-Ø15Ø7–1 × SYN-IR162-4 × MONØØ6Ø3–6 × DAS-4Ø278-9
4-event stack	MON 89034 × 1507 × MIR162 × NK603	MON-89Ø34-3 × DAS-Ø15Ø7–1 × SYN-IR162-4 × MONØØ6Ø3–6
	MON 89034 × 1507 × MIR162 × DAS-40278-9	MON-89Ø34-3 × DAS-Ø15Ø7–1 × SYN-IR162-4 × DAS-4Ø278-9
	MON 89034 × 1507 × NK603 × DAS-40278-9	MON-89Ø34-3 × DAS-Ø15Ø7–1 × MONØØ6Ø3–6 × DAS-4Ø278-9
	MON 89034 × MIR162 × NK603 × DAS-40278-9	MON-89Ø34-3 × SYN-IR162-4 × MONØØ6Ø3–6 × DAS-4Ø278-9
	1507 × MIR162 × NK603 × DAS-40278-9	DAS-Ø15Ø7–1 × SYN-IR162-4 × MONØØ6Ø3–6 × DAS-4Ø278-9
3-event stack	MON 89034 × 1507 × MIR162	MON-89Ø34-3 × DAS-Ø15Ø7–1 × SYN-IR162-4
	MON 89034 × 1507 × NK603	MON-89Ø34-3 × DAS-Ø15Ø7–1 × MONØØ6Ø3–6
	MON 89034 × 1507 × DAS-40278-9	MON-89Ø34-3 × DAS-Ø15Ø7–1 × DAS-4Ø278-9
	MON 89034 × MIR162 × NK603	MON-89Ø34-3 × SYN-IR162-4 × MONØØ6Ø3–6
	MON 89034 × MIR162 × DAS-40278-9	MON-89Ø34-3 × SYN-IR162-4 × DAS-4Ø278-9
	MON 89034 × NK603 × DAS-40278-9	MON-89Ø34-3 × MONØØ6Ø3–6 × DAS-4Ø278-9
	1507 × MIR162 × NK603	DAS-Ø15Ø7–1 × SYN-IR162-4 × MONØØ6Ø3–6
	1507 × MIR162 × DAS-40278-9	DAS-Ø15Ø7–1 × SYN-IR162-4 × DAS-4Ø278-9
	1507 × NK603 × DAS-40278-9	DAS-Ø15Ø7–1 × MONØØ6Ø3–6 × DAS-4Ø278-9
	MIR162 × NK603 × DAS-40278-9	SYN-IR162-4 × MONØØ6Ø3–6 × DAS-4Ø278-9
2-event stack	MON 89034 × 1507	MON-89Ø34-3 × DAS-Ø15Ø7–1
	MON 89034 × MIR162	MON-89Ø34-3 × SYN-IR162-4
	MON 89034 × NK603	MON-89Ø34-3 × MONØØ6Ø3–6
	MON 89034 × DAS-40278-9	MON-89Ø34-3 × DAS-4Ø278-9
	1507 × MIR162	DAS-Ø15Ø7–1 × SYN-IR162-
	1507 × NK603	DAS-Ø15Ø7–1 × MONØØ6Ø3–6
	1507 × DAS-40278-9	DAS-Ø15Ø7–1 × DAS-4Ø278-9

Degree of stacking	Event	Unique identifiers
	MIR162 × NK603	SYN-IR162-4 × MONØØ6Ø3–6
	MIR162 × DAS-40278-9	SYN-IR162-4 × DAS-4Ø278-9
	NK603 × DAS-40278-9	MONØØ6Ø3–6 × DAS-4Ø278-9

The term 'subcombination' refers to any combination of up to four of the maize events MON 89034, 1507, MIR162, NK603 and DAS-40278-9.

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

The safety of subcombinations occurring as segregating progeny in harvested grains of maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 is evaluated in the context of the assessment of the five-event stack maize in Section 3.5 of the present scientific opinion.

Maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 was developed by crossing the single lines MON 89034 (expressing Cry1A.105 and Cry2Ab2), 1507 (expressing Cry1F and PAT), MIR162 (expressing Vip3Aa20 and PMI), NK603 (expressing CP4 EPSPS) and DAS-40278-9 (expressing AAD-1) to confer resistance to certain lepidopteran pests and tolerance to glyphosate, glufosinate-ammonium, 2,4-D and the AOPP based herbicides.

All five single events, nine two-event stacks, six three-event stacks and one four-event stack were assessed previously (see Table 2) and no concerns for human and animal health or environmental safety were identified.

Table 2: Single maize events and subcombination of maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 previously assessed by the GMO Panel

Event	Application	EFSA Scientific Opinion
MON 89034	EFSA-GMO-NL-2007-37	EFSA (2008)
	EFSA-GMO-RX-015	EFSA GMO Panel (2019a)
1507	EFSA-Q-2004-011	EFSA (2004a)
	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 (2017b)
MIR162	EFSA-GMO-DE-2010-82	EFSA GMO Panel (2012)
NK603	EFSA-Q-2003-002	EFSA (2004b)
	EFSA-Q-2003-003	EFSA (2007)
	EFSA-GMO-NL-2005-22	EFSA (2009b)
	EFSA-GMO-RX-NK603	EFSA (2009b)
DAS-40278-9	EFSA-GMO-NL-2010-89	EFSA GMO Panel (2016)
1507 × NK603	EFSA-GMO-UK-2004-05	EFSA (2006)
	EFSA-GMO-RX-008	EFSA GMO Panel (2018a)
	EFSA-GMO-NL-2015-127	EFSA GMO Panel (2021a)
1507 × MON 89034	EFSA-GMO-CZ-2008-62	EFSA GMO Panel (2010c, 2011c)
	EFSA-GMO-NL-2017-139	EFSA GMO Panel (2021b)
1507 × MIR162	EFSA-GMO-DE-2010-86	EFSA GMO Panel (2018b)
	EFSA-GMO-NL-2017-139	EFSA GMO Panel (2021b)
1507 × DAS-40278-9	EFSA-GMO-NL-2013-112	EFSA GMO Panel (2019b)
	EFSA-GMO-NL-2013-113	EFSA GMO Panel (2019c)
MON 89034 × NK603	EFSA-GMO-NL-2007-38	EFSA GMO Panel (2009)
	EFSA-GMO-NL-2016-134	EFSA GMO Panel (2019d)
MON 89034 × DAS-40278-9	EFSA-GMO- NL-2013-112	EFSA GMO Panel (2019b)

Event	Application	EFSA Scientific Opinion
	EFSA-GMO- NL-2013-113	EFSA GMO Panel (2019c)
MON 89034 × MIR162	EFSA-GMO-NL-2016-131	EFSA GMO Panel (2019e)
	EFSA-GMO-NL-2016-134	EFSA GMO Panel (2019d)
	EFSA-GMO-NL-2017–144	EFSA GMO Panel (2019f)
NK603 × MIR162	EFSA-GMO-NL-2016-131	EFSA GMO Panel (2019e)
	EFSA-GMO-NL-2016-134	EFSA GMO Panel (2019d)
	EFSA-GMO-NL-2015-127	EFSA GMO Panel (2021a)
NK603 × DAS-40278-9	EFSA-GMO-NL-2013-112	EFSA GMO Panel (2019b)
	EFSA-GMO-NL-2019–164	EFSA GMO Panel (2021c)
1507 × NK603 × MON 89034	EFSA-GMO-NL-2009-65	EFSA GMO Panel (2010d, 2011d)
	EFSA-GMO-NL-2013-112	EFSA GMO Panel (2019b)
1507 × NK603 × MIR162	EFSA-GMO-NL-2015-127	EFSA GMO Panel (2021a)
1507 × NK603 × DAS-40278-9	EFSA-GMO-NL-2013-112	EFSA GMO Panel (2019b)
1507 × MON 89034 × DAS-40278-9	EFSA-GMO-NL-2013-112	EFSA GMO Panel (2019b)
	EFSA-GMO- NL-2013-113	EFSA GMO Panel (2019c)
NK603 × MON 89034 × MIR162	EFSA-GMO-NL-2016-131	EFSA GMO Panel (2019e)
	EFSA-GMO-NL-2016-134	EFSA GMO Panel (2019d)
NK603 × MON 89034 × DAS-40278-9	EFSA-GMO-NL-2013-112	EFSA GMO Panel (2019b)
MON 89034 × 1507 × NK603 × DAS-40278-9	EFSA-GMO-NL-2013-112	EFSA GMO Panel (2019b)

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 five single events has been reported by the applicant.

The applicant clarified that the maize 1507 sequence reported for the five-event stack maize contained one silent nucleotide change in the insert sequence compared to the corrected original sequence (EFSA GMO Panel, 2017b), that has already been assessed in the frame of previous applications (EFSA GMO Panel, 2018a,b, 2019b,c, 2021b). 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, 2017b). Analysis of the corrected sequencing data and the bioinformatic analyses performed on this sequence did not give rise to safety issues.

Updated bioinformatic analyses for events MON 89034, 1507, MIR162, NK603 and DAS-40278-9 confirmed that no known endogenous genes were disrupted by any of the inserts.

Updated bioinformatic analyses of the amino acid sequence of the newly expressed proteins Cry1A.105, Cry2Ab2, Cry1F, Vip3Aa20, PAT, CP4 EPSPS, AAD-1 and PMI confirmed previous results indicating no significant similarities to known toxins and allergens. Updated bioinformatic analyses of the newly created open reading frames (ORFs) within the inserts or spanning the junctions between the insert and the flanking regions for events MON 89034, 1507, MIR162, NK603 and DAS-40278-9 confirms that the production of a new peptide with significant similarity to toxins or allergens is highly unlikely.

In order to assess the possibility for horizontal gene transfer (HGT) by homologous recombination (HR), the applicant performed a sequence identity analysis for events MON 89034, 1507, MIR162, NK603 and DAS-40278-9 to microbial DNA. The likelihood and potential consequences of plant-to-bacteria gene transfer are described in Section 3.4.4.2.

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

3.3. Systematic literature review[5]

The GMO Panel assessed the applicant's literature searches on maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9, which include a scoping review, according to the guidelines given in EFSA (2010, 2019b).

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-2018-151. Based on the outcome of the scoping review, the GMO Panel agrees that there is limited value of undertaking a systematic review for maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 at present.

The performed literature searches are acceptable. The GMO Panel concludes that future searches on maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 must be fully conducted according to the guidelines given in EFSA (2019b).

The literature searches identified 12 relevant publications on maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9. Based on the relevant publications identified through the literature searches (Appendix B), the GMO Panel does not identify any safety issues pertaining to the intended uses of maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9.

3.4. Risk assessment of the five-event stack maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9

3.4.1. Molecular characterisation[6]

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 biological function of the inserts

Maize events MON 89034, 1507, MIR162, NK603 and DAS-40278-9 were combined by crossing to produce the five-event stack maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9. The structure of the inserts introduced into the five-event stack maize is described in detail in the respective EFSA scientific opinions (Table 2) and no new genetic modifications were involved. Genetic elements in the expression cassettes of the single events are summarised in Table 3.

The intended effects of the inserts in maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 are summarised in Table 4. Based on the known biological function of the newly expressed proteins (Table 4), the only foreseeable interactions at the biological level are among the Cry proteins or among the Vip3Aa20 and the Cry proteins in susceptible insects, which will be addressed in Section 3.4.4.

Table 3: Genetic elements in the expression cassettes of the events stacked in maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
MON 89034	35S (CaMV)	CAB (*Triticum aestivum*)	–	*cry1A.105** (*Bacillus thuringiensis*)	Hsp17 (*T. aestivum*)
	35S (FMV)		CTP (*Zea mays*)	*cry2Ab2** (*B. thuringiensis*)	nos (*Agrobacterium tumefaciens*)
1507	ubiZM1 (*Z. mays*)		–	*cry1F** (*B. thuringiensis* sbsp. *aizawai*)	ORF25 (*A. tumefaciens*)
	35S (CaMV)	–	–	*pat** (*Streptomyces viridochromogenes*)	35S (CaMV)

[5] Dossier: Part II – Section 7 and additional information 12/5/2022.
[6] Dossier: Part II – Section 1.2; additional information: 18/3/2019, 25/3/2019, 11/11/2019, 11/2/2022 and 8/6/2022.

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
MIR162	ZmUbiInt (*Z. mays*)	–	–	*vip3Aa20** (*B. thuringiensis* strain AB88)	35S (CaMV)
	ZmUbiInt (*Z. mays*)	–	–	*pmi* (*Escherichia coli* strain K-12)	nos (*A. tumefaciens*)
NK603	ract1 (*Oryza sativa*)	ract1 (*O. sativa*)	CTP2 (*Arabidopsis thaliana*)	*cp4 epsps** (*A. tumefaciens* strain CP4)	nos (*A. tumefaciens*)
	35S (CaMV)	I-Hsp70 (*Z. mays*)	CTP2 (*A. thaliana*)	*cp4 epsps l214p** (*A. tumefaciens* strain CP4)	nos (*A. tumefaciens*)
DAS-40278-9	ZmUbi1 (*Z. mays*)	–	–	*aad-1** (*Sphingobium herbicidovorans*)	ZmPer5 (*Z. mays*)

CaMV: cauliflower mosaic virus; FMV: figwort mosaic virus.
–: When no element was specifically introduced to optimise expression.
*: Codon optimised for plant expression.

Table 4: Characteristics and intended effects of the events stacked in maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9

Event	Protein	Donor organism and biological function	Intended effects in GM plant
MON 89034	Cry1A.105	Based on genes from *Bacillus thuringiensis* subsp. *kurstaki* and subsp. *aizawai*. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes (Schnepf et al., 1998, Ellis et al., 2002)	Event 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 genes 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 Phosphinothricin-acetyl-transferase (PAT) enzyme acetylates L-glufosinate-ammonium (Thompson et al., 1987; Wohlleben et al., 1988; Eckes et al., 1989)	Event 1507 expresses the PAT protein which confers tolerance to glufosinate ammonium-based herbicides (Droge-Laser et al., 1994)
MIR162	Vip3Aa20	Based on a gene from *Bacillus thuringiensis* strain AB88 (Estruch et al., 1996). In addition to Cry proteins, *B. thuringiensis* also produces insecticidal proteins during its vegetative growth stage. These are referred to as vegetative insecticidal proteins (Fang et al., 2007).	Event MIR162 expresses a modified version of the *B. thuringiensis vip3Aa1* gene, and encodes Vip3Aa20, a protein toxic to certain lepidopteran larvae feeding on maize.

Event	Protein	Donor organism and biological function	Intended effects in GM plant
	PMI	Based on a gene from *E. coli*. The phosphomannose isomerase (PMI) catalyses the isomerisation of mannose-6-phosphate to fructose-6-phosphate and plays a role in the metabolism of mannose (Markovitz et al., 1967).	Event MIR162 expresses PMI, which is used as selectable marker. Mannose normally inhibits root growth, respiration and germination. Transformed cells expressing PMI are able to utilise mannose as a carbon source (Negrotto et al., 2000).
NK603	CP4 EPSPS	Based on a gene from *Agrobacterium* strain CP4 (Barry et al., 2001). 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) is an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Herrmann, 1995).	Event NK603 expresses the bacterial CP4 EPSPS protein which confers tolerance to glyphosate-containing herbicides as it has lower affinity towards glyphosate than the plant endogenous enzyme.
	CP4 EPSPS L214P	Based on a gene from *Agrobacterium* strain CP4 (Barry et al., 2001). 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) is an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Herrmann, 1995).	Event NK603 expresses a modified version of the bacterial CP4 EPSPS protein which confers tolerance to glyphosate-containing herbicides as it has lower affinity towards glyphosate than the plant endogenous enzyme.
DAS-40278-9	AAD-1	Based on a gene from *Sphingobium herbicidovorans*. Aryloyankanoate dioxygenase (AAD-1) facilitates the breakdown of phenoxy auxin and aryloxyphenoxypropionate herbicides (AOPP) (Wright et al., 2009).	Event DAS-40278-9 expresses AAD-1 protein which degrades the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and AOPP thus conferring tolerance to these herbicides.

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

The genetic stability of the inserted DNA over multiple generations in the MON 89034, 1507, MIR162, NK603 and DAS-40278-9 single maize events was demonstrated previously (Table 2, Section 2.2). Integrity of these events in maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 was demonstrated by Southern analyses. In addition, the sequence of the events (inserts and their flanking regions) was determined in the five-event stack maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 and compared to the sequences originally reported for the five single events. The sequences of the events in the five-event stack maize are identical to the sequences already assessed (see Table 2 and Section 2.2) for the five single events, thus confirming that the integrity of these events was maintained in the five-event stack maize.

3.4.1.3. Information on the expression of the inserts

Cry1A.105, Cry2Ab2, Cry1F, Vip3Aa20, PAT, CP4 EPSPS, AAD-1 and PMI protein levels were analysed by enzyme-linked immunosorbent assay (ELISA) in material harvested from a field trial across eight locations in Argentina in 2015–2016. Samples analysed included leaves (V2-V4, V9 and R1), roots (R1), pollen (R1), forage (R5) and grains (R6), both those treated and not treated with glyphosate, glufosinate-ammonium, haloxyfop[7] and 2,4-D containing herbicides.

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

The levels of all the newly expressed proteins in the five-event maize stack and the corresponding singles were comparable in all tissues (Appendix C). Therefore, there is no indication of an interaction that may affect the levels of the newly expressed proteins in this stack.

[7] Haloxyfop belongs to the AOPP chemical group of herbicides.

3.4.1.4. Conclusion on molecular characterisation

The molecular data establish that the events stacked in maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins are similar in the five-event stack and in the single events. 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 4) of the newly expressed proteins, the only potential functional interactions are among the Cry and Vip proteins in susceptible insects which will be dealt with in Sections 3.4.4 and 3.5.2.3.

3.4.2. Comparative analysis[8]

3.4.2.1. Overview of studies conducted for the comparative analysis

Application EFSA-GMO-NL-2018-151 presents data on agronomic and phenotypic characteristics, as well as on forage and grain composition of five-event stack maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 (Table 5 and Appendix A).

Table 5: Main comparative analysis studies to characterise five-event stack maize provided in the application EFSA-GMO-NL-2018-151

Study focus	Study details	Comparator	Non-GM reference varieties
Agronomic and phenotypic analysis	Field study, USA, 2020, ten sites(a)	SLB01 × PH184C	20(b)
Compositional analysis	Field study, USA, 2020, eight sites(a)		

GM: Genetically modified.

(a): The field trials were located: two in Iowa, two in Illinois and one in Indiana, Minnesota, Nebraska, Pennsylvania. Two additional sites used only for agronomic and phenotypic analysis were included and located in USA: one in Texas and one in Wisconsin.

(b): Non-GM hybrid maize with their corresponding comparative relative maturity indicated in brackets were BK5337 (103), PB5385 (103), BK5433 (104), PB5466 (104), PB5624 (105), XL5513 (105), P0506 (105), 35A52 (106), P0604 (106), PB5646 (106), MPSMY06R30 (106), MPS2R602 (106), P0574 (106), P0760 (107), BK5883 (108), P0843 (108), BKXL-5858 (108), MPSMY09V40 (109), XL5939 (109), P0928 (109).

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 five-event stack maize not exposed to the intended herbicide, the five-event stack maize exposed to the intended herbicides, the comparator SLB01 × PH184C and four non-GM reference varieties.

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

3.4.2.3. Suitability of selected test materials

3.4.2.3.1. Selection of the test materials

To obtain the five-event stack maize, the single events MON 89034, 1507, MIR162, NK603 and DAS-40278-9 were transferred in the genetic background of two different non-GM maize inbred lines, maize SLB01 and maize PH184C. In subsequent subsections, maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 refers to hybrid (F_1 generation) obtained crossing GM inbred line SLB01

[8] Dossier: Part II – Section 1.3; additional information: 11/11/2019, 28/7/2021, 17/11/2021, 11/2/2022 and 2/3/2022.

[9] In detail, the four outcomes are category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence).

(carrying events MON 89034, 1507, NK603 and DAS-40278-9) with GM inbred line PH184C (carrying MIR162).

The comparator used in the field trials is the non-GM maize hybrid SLB01 × PH184C, which has the similar genetic background as maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 (as documented by the additional information), and is considered to be a suitable comparator.

The five-event stack maize and the comparator, both with a comparative relative maturity (CRM) of 105–106, which is considered appropriate for growing in environments across USA, where the comparative field trials were conducted.

Commercial non-GM reference varieties with a CRM ranging from 103 to 109 were selected by the applicant and, at each selected site, four reference varieties were tested (see Table 2). On the basis of the provided information on relative maturity classes and year of commercialisation, the GMO Panel considers the selected non-GM reference varieties appropriate for the comparative assessment.

3.4.2.3.2. Seed production and quality

Seeds of five-event stack maize and the comparator used in the 2020 field trials were produced from plants harvested and stored under similar conditions, before being sown in the field trial sites. The seed lots were verified for their identity via event specific quantitative polymerase chain reaction analysis.

The grains were tested for their germination capacity under warm and cold temperature conditions.[10] Germination capacity of the GM five-event stack maize was compared with the one of its comparator and the results[11] of these studies indicate that the seed germination of five-event stack maize was not different than that of its comparator.

3.4.2.3.3. Conclusion on suitability

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

3.4.2.4. Representativeness of the receiving environments

3.4.2.4.1. Selection of field trial sites

The selected field trials sites were located in commercial maize-growing regions of the United States of America. The soil and climatic characteristics of the selected fields were diverse,[12] corresponding to optimal, near-optimal and suboptimal conditions for maize cultivation (Sys et al., 1993).

The GMO Panel considers that the selected sites, including the subset chosen for the compositional analysis, reflect commercial maize-growing regions in which the test materials are likely to be grown.

3.4.2.4.2. Meteorological conditions

Maximum and minimum mean temperatures and sum of precipitations were provided on a weekly basis. Some exceptional weather conditions were reported at six of the selected sites.[13] However, due to the lack of major impacts on plant growth at these sites, the GMO Panel considers that the exceptional weather conditions did not invalidate the selection of the field trial sites for the comparative analysis.

3.4.2.4.3. Management practices

The field trials included plots containing five-event stack maize, plots with the comparator and plots with non-GM maize reference varieties, managed according to local agricultural practices. In addition, the field trials included plots containing five-event stack maize managed following the same

[10] The seed germination test report was produced following the International Rules for Seed Testing (ISTA, 2019). Warm temperature condition corresponds to 25°C and 90% relative humidity for 7 days and cold temperature to 10°C and 90% relative humidity for 7 days followed by 5 days at 25°C and 90% relative humidity.

[11] The GM hybrid maize and the comparator showed a mean germination of 99% under both warm and cold temperature conditions.

[12] Soil types of the field trials were sandy clay loam, silty clay loam, clay loam, silt loam; soil organic matter ranged from 1.0% to 2.7%; pH ranged from 5.8 to 7.2; average temperatures and sum of precipitations during the usual crop growing season ranged, respectively, from 16.2°C to 23.9°C and from 309 mm to 791 mm.

[13] Windstorm events were registered at one field trial in Illinois, Minnesota and Pennsylvania; hail was recorded at another field trial in Illinois; heavy rain and windstorm at one field trial in Iowa and excessive rainfall at one field trial in Indiana.

agricultural practices, but conventional herbicides were replaced with the intended quizalofop-containing herbicide[14] that was applied at BBCH 12 growth stage, while glyphosate-, 2,4-D- and glufosinate-ammonium-containing herbicides were applied at BBCH 14 growth stage.

The GMO Panel considers that the management practices, including sowing, harvesting and application of plant protection products, were appropriate for the selected receiving environments.

3.4.2.4.4. Conclusion on representativeness

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

3.4.2.5. Agronomic and phenotypic analysis

Eleven agronomic and phenotypic endpoints[15] plus information on abiotic stressors, disease incidence and arthropod damage were collected from the field trial sites (see Table 5). The endpoint ear count and dropped ears were not analysed with formal statistical methods because of lack of variability in the data.

The statistical analysis (Section 3.4.2.2) was applied to eight endpoints, with the following results:

- For the five-event stack maize (treated with conventional herbicides), the test of difference identified statistically significant differences with the comparator for early stand count, plant height, final stand count and 100-kernel weight. All these endpoints fell under equivalence category I.
- For the five-event stack maize (treated with the intended herbicides), the test of difference identified statistically significant differences with the comparator for plant height and 100-kernel weight. All these endpoints fell under equivalence category I.

3.4.2.6. Compositional analysis

Forage and grain of maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 harvested from the field trials (Table 5) were analysed for 80 constituents (10 in forage and 70 in grain), including those recommended by OECD (OECD, 2002). The statistical analysis as described in Section 3.4.2.2 was not applied to nine grain constituents[16] because their concentration in more than half of the samples was below the limit of quantification.

The statistical analysis was applied to a total of 71 constituents (10 in forage[17] and 61 in grain[18]); a summary of the outcome of the test of difference and the test of equivalence is presented in Table 6:

- For the five-event stack maize not treated with the intended herbicides, statistically significant differences with the comparator were found for 32 endpoints (one in forage and 31 in grains). All these endpoints fell under equivalence category I or II except for histidine which fell under equivalence category III and ash, behenic acid (C22:0), arginine, glycine, lysine, phosphorus, potassium and phytic acid which fell under equivalence category IV, all in grain (Table 7).
- For the five-event stack maize treated with the intended herbicides, statistically significant differences with the comparator were found for 39 endpoints (one in forage and 38 in grains). All these endpoints fell under equivalence category I or II except for histidine which fell under equivalence category III and ash, behenic acid (C22:0), arginine, glycine, phosphorus, potassium and phytic acid which fell under equivalence category IV, all in grain (Table 7).

[14] Quizalofop belongs to the AOPP chemical group of herbicides.

[15] Early stand count, days to flowering, plant height, days to maturity, total lodging, final stand count, ear count, dropped ears, yield, harvest grain moisture and 100-kernel weight.

[16] Lauric acid (C12:0), myristic acid (C14:0), heptadecanoic acid (C17:0), heptadecenoic acid (C17:1), eicosadienoic acid (C20:2), riboflavin, β-tocopherol, δ-tocopherol and furfural.

[17] Moisture, crude protein, crude fat, ash, carbohydrates, crude fibre, acid detergent fibre (ADF), neutral detergent fibre (NDF), calcium and phosphorus.

[18] Proximates and fibre content (ash, carbohydrates, crude fat, crude fibre, crude protein, moisture, ADF, NDF and total dietary fibre), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, zinc), vitamins (α-tocopherol, ß-carotene, γ-tocopherol, total tocopherols, thiamine, niacin, pantothenic acid, pyridoxine, folic acid), 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), α-linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C20:1), behenic acid (C22:0), lignoceric acid (C24:0)) and other compounds (ferulic acid, inositol, p-coumaric acid, phytic acid, raffinose and trypsin inhibitor).

Table 6: Outcome of the comparative compositional analysis in grain and forage for five-event stack maize. The table shows the number of endpoints in each category

		Test of difference[a]			
		Treated[c]		Not-treated[c]	
		Not different	Significantly different	Not different	Significantly different
Test of equivalence[b]	Category I/II	34	23[d]	28	30[d]
	Category III/IV	3[e]	9[f]	3[e]	8[f]
	Not categorised	2[g]	–	1[g]	1[h]
	Total endpoints	71		71	

(a): Comparison between the five-event stack maize and its comparator.
(b): Four different outcomes: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence). Not categorised means that the test of equivalence was not applied because of the lack of variation among the non-GM reference varieties.
(c): Treated/not treated with the intended herbicides.
(d): Endpoints with significant differences between the five-event stack maize and its comparator and falling under equivalence category I-II. For forage, both treated and not treated: calcium. For grains, not treated only: none. Treated only: total dietary fibre, ADF, palmitoleic acid (C16:1), lignoceric acid (C24:0), magnesium, folic acid and inositol. Both treated and not treated: moisture, carbohydrates, crude protein, oleic acid (C18:1), linoleic acid (C18:2), α-linolenic acid (C18:3), arachidic acid (C20:0), alanine, aspartic acid, glutamic acid, isoleucine, leucine, phenylalanine, proline, serine, threonine, tyrosine, valine, calcium, zinc, β-carotene and raffinose.
(e): Endpoints with no significant differences between the five-event stack maize and its comparator and falling in equivalence category III/IV. In forage, none. In grain, not treated only: stearic acid (C18:0). Treated only: lysine. Both treated and not treated: tryptophan, thiamine.
(f): Endpoints with significant differences between the five-event stack maize and its comparator and falling in equivalence category III/IV. In forage, none. In grain, not treated only: lysine. Treated only: none. Both not treated and treated: ash, behenic acid (C22:0), arginine, glycine, histidine, phosphorus, potassium and phytic acid. 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 five-event stack maize and its comparator. In forage, none. In grain, not treated only: pyridoxine. Treated only: none. Both not treated and treated: sodium.
(h): Endpoints that were not categorised for equivalence and for which significant differences were identified between the five-event stack maize and its comparator: pyridoxine in grain (treated only). Quantitative results for this endpoint are reported in Table 7.

The GMO Panel assessed all the significant differences between the five-event stack maize and its 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 five-event stack maize and its comparator and falling under equivalence category III/IV are given in Table 7, together with endpoints with significant differences with the conventional counterpart where the equivalence test was not applied because of the lack of variation among the non-GM varieties.

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

Endpoint	Maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9		Comparator	Non-GM reference varieties	
	Not treated[a]	Treated[a]		Mean	Equivalence limits
Ash (% dw)	1.49*	1.46*	1.38	1.24	1.16–1.33
Behenic acid (C22:0) (% FA)	0.278*	0.283*	0.270	0.222	0.184–0.260
Arginine (% dw)	0.469*	0.462*	0.449	0.409	0.372–0.447
Glycine (% dw)	0.427*	0.427*	0.415	0.387	0.357–0.417
Histidine (% dw)	0.341*	0.340*	0.325	0.312	0.285–0.339

Endpoint	Maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9		Comparator	Non-GM reference varieties	
	Not treated[(a)]	Treated[(a)]		Mean	Equivalence limits
Lysine (% dw)	0.332*	0.332	0.326	0.293	0.264–0.323
Phosphorus (% dw)	0.365*	0.371*	0.339	0.311	0.288–0.335
Potassium (% dw)	0.413*	0.421*	0.389	0.354	0.318–0.390
Pyridoxine (mg/kg dw)	5.24	4.88*	5.32	4.81	–[(b)]
Phytic acid (% dw)	1.07*	1.06*	0.949	0.878	0.783–0.972

dw: dry weight; % FA: percentage total fatty acids.
For the five-event stack maize, significantly different values are marked with an asterisk, while the outcomes of the test of equivalence are differentiated by greyscale backgrounds: white for equivalence category I or II and for pyridoxine for which the test of equivalence was not applied; light grey (equivalence category III); and dark grey (equivalence category IV).
(a): Treated with the intended herbicides quizalofop[14] and a mixture of glufosinate-ammonium, glyphosate and 2,4-D.
(b): Test of equivalence not applied because of the lack of variation among the non-GM reference varieties.

3.4.2.7. Conclusion on comparative analysis

Considering the selection of test materials, the field trial sites and the associated management practices and the agronomic-phenotypic characterisation as an indicator of the overall field trial quality, the GMO Panel concludes that the field trials were appropriate to support 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 tested between the five-event stack maize and the comparator needs further assessment regarding their potential environmental impact.
- None of the differences identified in forage and grain composition between the five-event stack maize and the comparator needs further assessment regarding food and feed safety except for the levels in grain of: ash, behenic acid (C22:0), arginine, glycine, histidine, phosphorus, potassium, phytic acid (both treated and not treated), lysine (not treated) and pyridoxine (treated), which are further assessed in Section 3.4.3.

3.4.3. Food/feed safety assessment[19]

3.4.3.1. Effects of processing

The five-event stack maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 will undergo existing production processes used for conventional maize. No novel production process is envisaged. Based on the outcome of the comparative assessment, processing of the five-event stack maize 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. Stability of newly expressed proteins

Protein stability is one of several relevant parameters to consider in the weight-of-evidence approach in protein safety assessment (EFSA GMO Panel, 2010e, 2011a, 2017a, 2021e). The term protein stability encompasses several properties such as thermal stability, pH-dependent stability, proteolytic stability and physical stability (e.g. tendency to aggregate), among others (Li et al., 2019). It has been shown, for example, that when characteristics of known food allergens are examined, one prominent trait attributed to food allergens is protein stability (Helm, 2001; Breiteneder and Mills, 2005; Costa et al., 2022).

[19] Dossier: Part II – Sections 1.4, 1.5, 1.6, 2; additional information: 17/6/19, 11/11/2019, 16/7/20, 12/2/2021, 9/7/2021, 17/11/21 and 11/2/2022.

3.4.3.2.1. Effect of temperature and pH on newly expressed proteins

The effects of temperature and pH on the Cry1A.105, Cry2Ab2, Cry1F, Vip3Aa20, PAT, CP4 EPSPS, AAD-1 and PMI proteins have been previously evaluated by the GMO Panel (Table 2). No new information has been provided in the context of this application.

3.4.3.2.2. In vitro protein degradation by proteolytic enzymes

The resistance to degradation by pepsin of the newly expressed Cry1A.105, Cry2Ab2, Cry1F, Vip3Aa20, PAT, CP4 EPSPS, AAD-1 and PMI proteins has been previously evaluated by the GMO Panel (Table 2). No new information has been provided in the context of this application.

3.4.3.3. Toxicology

3.4.3.3.1. Testing of newly expressed proteins

Eight proteins (Cry1A.105, Cry2Ab2, Cry1F, Vip3Aa20, PAT, CP4 EPSPS, AAD-1 and PMI) are newly expressed in the five-event stack maize (Section 3.4.1). The GMO Panel has previously assessed these proteins in the context of the single maize events (Table 2), and no safety concerns were identified for humans and animals (i.e. farmed and companion 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 among the proteins newly expressed in the five-event stack maize has been assessed with regard to human and animal health.

The three insecticidal proteins Cry1A.105, Cry2Ab2 and Cry1F are delta-endotoxins acting through cellular receptors found in target insect species. It is reported that the gastrointestinal tract of mammals, including humans, lacks receptors with high specific affinity to Cry proteins (Hammond et al., 2013; Koch et al., 2015; Jurat-Fuentes and Crickmore, 2017).

The Vip3Aa20 protein is a protein secreted by *Bacillus thuringiensis* during its vegetative phase acting in target insects via a mechanism similar to that of Cry proteins (Chakroun et al., 2016; Bel et al., 2017). The four enzymatic proteins (PMI, PAT, CP4 EPSPS and AAD-1) catalyse distinct biochemical reactions, acting on unrelated substrates and are not expected to interact. The PMI enzyme catalyses the reversible interconversion of mannose 6-phosphate and fructose 6-phosphate. The CP4 EPSPS acts on the shikimic acid pathway for the biosynthesis of aromatic amino acids in plants, showing high substrate specificity. The PAT enzyme acts on the glufosinate-ammonium-based herbicides and AAD-1 enzyme degrades 2,4-D and AOPP class of herbicides. On the basis of the known biological function of the individual newly expressed proteins (Table 4), there is currently no expectation for their possible interactions relevant to the food and feed safety of this five-event stack maize.

The GMO Panel concludes that there are no safety concerns to human and animal health related to the newly expressed proteins Cry1A.105, Cry2Ab2, Cry1F, Vip3Aa20, PAT, CP4 EPSPS, AAD-1 and PMI in the five-event stack maize.

3.4.3.3.2. Testing of new constituent other than proteins

Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, no new constituents other than newly expressed proteins have been identified in seed and forage from five-event stack maize. Therefore, no further food/feed safety assessment of components other than the newly expressed proteins is required.

3.4.3.3.3. Information on altered levels of food and feed constituents

No altered levels of food/feed constituents have been identified in seed and forage from the five-event stack maize except for ash, behenic acid (C22:0), arginine, glycine, histidine, phosphorus, potassium, phytic acid (both treated and not treated), lysine (not treated) and pyridoxine (treated). These changes are considered not to represent a toxicological concern, considering the biological role of the affected constituent and the magnitude of the changes; therefore, no further toxicological assessment is needed. Further information on the relevance of these findings is provided in Section 3.4.3.6.

3.4.3.3.4. Testing of the whole genetically modified food and feed

Based on the outcome of the molecular characterisation, comparative analysis and toxicological assessment, no indication of findings relevant to food/feed safety related to the stability and expression of the inserts or to interaction between the transformation events, and no modifications of

toxicological concern in the composition of the five-event stack maize have been identified (see Sections 3.4.1, 3.4.2 and 3.4.3.3). Therefore, animal studies on food/feed derived from this five-stack maize are not necessary (EFSA GMO Panel, 2011a). In accordance to Regulation (EU) No 503/2013, the applicant provided a 90-day oral repeated-dose toxicity study in rats on whole food and feed from each of the maize single event composing the five-event stack maize. The GMO Panel had previously concluded that these studies are in line with Regulation (EU) No 503/2013 and do not show adverse effects related to diets incorporating the single-event maize MON 89034 (EFSA GMO Panel, 2019e), 1507 (EFSA GMO Panel, 2021a,b), MIR162 (EFSA GMO Panel, 2019c), NK603 (EFSA GMO Panel, 2019c) and DAS-40278-9 (EFSA GMO Panel, 2021c).

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, 2017a).

3.4.3.4.1. Assessment of allergenicity of the newly expressed proteins

The GMO Panel has previously evaluated the safety of Cry1A.105, Cry2Ab2, Cry1F, Vip3Aa20, PAT, CP4 EPSPS, AAD-1 and PMI proteins individually, and no evidence of allergenicity was identified in the context of the applications assessed (Table 2). No new information on allergenicity of the proteins newly expressed in this five-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 five-event stack maize.

The GMO Panel has previously evaluated the safety of the newly expressed proteins, and no evidence of adjuvanticity was identified in the context of the applications assessed (Table 2). 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 five-event stack maize has similar levels of the individual Bt proteins as those in the respective single maize events (see Section 3.4.1.4). The GMO Panel did not find indications that the Bt proteins at the levels expressed in this five-event stack maize might be adjuvants able to enhance an allergic reaction.

The applicant also provided information on the safety of the Cry1A.105, Cry2Ab2, Cry1F, Vip3Aa20, PAT, CP4 EPSPS, AAD-1 and PMI proteins regarding their potential to cause a celiac disease response. For such assessment, the applicant followed the principles described in the EFSA GMO Panel guidance document (EFSA GMO Panel, 2017a). The assessment of the Cry2Ab2, Vip3Aa20, CP4 EPSPS (including its variant CP4 EPSPS L214P) and AAD-1 proteins identified no perfect or relevant partial matches with known celiac disease peptide sequences. The assessment of the Cry1F, Cry1A.105, PAT and PMI proteins revealed partial matches containing the Q/E-X1-P-X2 motif and required further investigations. Several of these partial matches have been previously assessed by the EFSA GMO Panel (2019d,g, 2021c,d). Based on additional considerations on position and nature of amino acids flanking the motifs, such as the presence of two consecutive prolines and the charge and size of adjacent amino acids (EFSA GMO Panel, 2017a), the relevant peptides containing the motif do not raise concern as they fail to mimic gluten sequences. Therefore, no indications of safety concern were identified by the GMO Panel.

3.4.3.4.2. Assessment of allergenicity of the whole GM plant or crop

The GMO Panel regularly reviews the available publications on food allergy to maize. However, maize is not considered a common allergenic food[20] (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

[20] 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.

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 five-event stack maize with respect to that derived from the non-GM comparator and the non-GM reference varieties tested.

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 Cry1A.105, Cry2Ab2, Cry1F, Vip3Aa20, PAT, CP4 EPSPS, AAD-1 and PMI proteins newly expressed in the five-event stack maize. Dietary exposure was estimated based on protein expression levels reported in this application for the five-event stack maize treated with glyphosate, glufosinate-ammonium, haloxyfop and 2,4-D, the current available consumption data and feed practices, the foods and feeds currently available on the market and the described processing conditions.

For the purpose of estimating dietary exposure, the levels of newly expressed proteins in the five-event stack maize grains, forage and pollen were derived from replicated field trials (four replicates from eight locations, n = 32) in 2015–2016 in Argentina. Table 8 describes the protein expression levels used to estimate both human and animal dietary exposure.

Table 8: Mean values (n = 32, μg/g dry weight and μg/g fresh weight) for newly expressed proteins in grains, forage and pollen from maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 treated with the intended herbicides[(a)]

Protein	Tissue/developmental stage		
	Grains/R6 (μg/g dry weight/fresh weight)	Pollen/R1 (μg/g dry weight)[(b)]	Forage/R4 (μg/g dry weight)
Cry1A.105	0.46/0.37	3.6	5.2
Cry2Ab2	8.5/6.8	5.0	84
Cry1F	4.1/3.2	21	4.9
PAT	< LOD/< LOD[(c)]	< LOD[(c)]	< LOD – 0.14[(d)]
Vip3Aa20	46/37	77	60
PMI	2.5/2.0	4.7	3.1
CP4 EPSPS	18/14	450	31
AAD-1	3.4/2.7	120	4.1

(a): Intended herbicides: haloxyfop[7], glufosinate, glyphosate and 2,4-dichlorophenoxyacetic acid (2,4-D).
(b): Concentrations values in pollen were adjusted to 6% moisture content before using them to estimate dietary exposure to the different newly expressed protein via the consumption of pollen supplements.
(c): All samples were below the limit of detection for PAT protein in grain (LOD = 0.025 μg/g dry weight and 0.020 μg/g fresh weight), for PAT protein in pollen (LOD = 0.025 μg/g dry weight).
(d): Limit of detection for PAT protein in forage (LOD = 0.025 μg/g dry weight).

3.4.3.5.1. Human dietary exposure

Chronic and acute dietary exposure to Cry1A.105, Cry2Ab2, Cry1F, Vip3Aa20, PAT, CP4 EPSPS, AAD-1 and PMI proteins newly expressed in the five-event stack maize were provided. The applicant followed the methodology described in the EFSA Statement 'Human dietary exposure assessment to newly expressed protein in GM foods' (EFSA, 2019a) to estimate human dietary exposure in average and high consumers making use of summary statistics of consumption.

Human dietary exposure was estimated across different European countries on different population groups: young population (infants, toddlers, 'other children'), adolescents, adult population (adults, elderly and very elderly) and special populations (pregnant and lactating women). Since no specific consumption data were available on commodities containing, consisting of or obtained from the five-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).[21] Corn oil, corn starch and corn syrup were excluded from the assessment since no proteins are expected to be present in these commodities.

[21] https://www.efsa.europa.eu/en/applications/gmo/tools. Data accessed: August 2019.

Mean protein expression values on fresh weight basis 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, 2019a). 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.[22] No losses in the newly expressed proteins during processing were considered, except for certain commodities excluded from the exposure estimations (corn oil, corn starch, corn syrup).

The highest acute dietary exposure (high consumers) was estimated in the age class 'Other children' with exposure estimates that ranged between 0.30 μg/kg body weight (bw) per day and 562 μg/kg bw per day for PAT and Vip3Aa20, respectively. The main average contributor to the exposure in the dietary survey with the highest estimates was corn grains.

The highest chronic dietary exposure (high consumers) was estimated in the age class 'Infants' with exposure estimates that ranged between 0.11 μg/kg bw per day and 209 μg/kg bw per day for PAT and Vip3Aa20, respectively. The main average contributor to the exposure in the dietary survey with the highest estimates was sweet corn.

An ad hoc dietary exposure scenario was carried out for consumers of pollen supplements under the assumption that these supplements might be made of pollen from the five-event stack maize. Consumption data on pollen supplements are available for few consumers across eight different European countries.[23] The low number of consumers available adds uncertainty to the exposure estimations which should be carefully interpreted, and it prevents from estimating exposure for high consumers of pollen supplements. In average consumers of pollen supplements, the highest acute dietary exposure would range from 0.014 μg/kg bw per day for PAT to 314 μg/kg bw per day for CP4 EPSPS, in the elderly population. Similarly, the highest chronic dietary exposure in average consumers would range from 0.009 μg/kg bw per day for PAT to 209 μg/kg bw per day for CP4 EPSPS, also in the elderly population.

3.4.3.5.2. Animal dietary exposure

Dietary exposure to Cry1A.105, Cry2Ab2, Cry1F, Vip3Aa20, PAT, CP4 EPSPS, AAD-1 and PMI proteins in the five-event stack maize 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 meal and forage). A conservative scenario with 100% replacement of conventional maize products by the five-event stack maize products was considered.

Mean levels (dry weight) of the newly expressed proteins in grains and forage from the five-event stack maize treated with the intended herbicide used for animal dietary exposure are listed in Table 8. All the grain samples analysed in the five-event stack maize for the presence of PAT protein were below the limit of detection (LOD = 0.025 μg/g dry weight). For forage samples, analytical results ranged from below the LOD and 0.14 μg/g dry weight. For estimating dietary exposure, the limit of detection (LOD = 0.025 μg/g dry weight) was used as the assumed mean amount of protein in grain.

The applicant estimated dietary exposure to Cry1A.105, Cry2Ab2, Cry1F, Vip3Aa20, PAT, CP4 EPSPS, AAD-1 and PMI proteins via the consumption of maize grains in chicken for fattening, laying hen, turkey for fattening, pig for fattening, sow lactating, cattle for fattening, dairy cow, sheep/goat, dog and cat, the consumption of maize gluten meal in salmon and the consumption of maize forage in laying hen, sow lactating, cattle for fattening and dairy cow.

The exposure was calculated for the select animals using estimates of daily feed intake (EFSA FEEDAP Panel, 2017) and maize grain, gluten meal and forage inclusion rates for the EU (OECD, 2013; FAO, 2017; and additional information[24]). Estimated dietary exposure in the concerned animals is reported in Appendix D.

3.4.3.6. Nutritional assessment of endogenous constituents

The intended traits of the five-event stack maize are herbicide tolerance and resistance to certain lepidopteran pests, with no intention to alter nutritional parameters. However, in maize grains, the levels of ash, behenic acid, arginine, glycine, histidine, phosphorus, potassium, phytic acid (all in both

[22] Example: 100 g of maize bread is 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 33.7 μg of Vip3Aa20 per gram of maize bread as compared to the 37 μg/g reported as mean concentration in the maize grains.

[23] https://www.efsa.europa.eu/en/food-consumption/comprehensive-database. Data accessed: December 2021.

[24] Additional information: 11/2/2022.

treated and not treated plants with the intended herbicides), lysine (not treated) and pyridoxine (treated) were significantly different from the comparator and showed a lack of equivalence with the set of non-GM reference varieties/could not be categorised (Section 3.4.2.6). The biological relevance of these compounds, the role of the five-event stack maize as contributor to their total intake and the magnitude and direction of the observed changes were considered during the nutritional assessment.

3.4.3.6.1. Human nutrition

Overall, maize protein is considered of low nutritional quality due to a poor balance of indispensable amino acids, in particular due to the low levels of lysine and tryptophan (Huang et al., 2004). Among the four amino acids being assessed, only histidine and lysine are considered as indispensable amino acids. For both amino acids, there is a small increase in the grains from the five-event stack maize as compared to the comparator, around 2% for lysine and 5% for histidine. Based on this information, the changes identified in the amino acid content do not represent a nutritional concern.

Potassium and phosphorus are the most abundant minerals in maize (Suri and Tanumihardjo, 2016). An increase between 6–8% and 8–9% was observed in the five-event stack maize as compared to the comparator for potassium and phosphorus, respectively. The increase in these two minerals probably explains the higher levels of ash observed as compared to the comparator. Potassium is an essential compound involved in many different physiological processes; no tolerable upper intake level (UL) has been set for potassium (EFSA NDA Panel, 2016a). Taken together, the reported small increase of potassium does not represent a nutritional concern. The increase in phosphorus is very likely related to the higher levels in phytic acid (assessed below) as the increase of this compound is of the same magnitude in the five-event stack maize.[25]

An increase of phytic acid (myo-inositol 1,2,3,4,5,6-hexakis-dihydrogen phosphate) content between 12% and 13% was observed in the grains from the five-event stack maize as compared to the comparator. Phytic acid is the primary storage form of phosphorus in seeds and provides protection against oxidative stress (OECD, 2002; Doria et al., 2009). In the context of human nutrition, phytic acid is typically considered as an antinutrient that reduces mineral bioavailability. There are many other dietary sources of phytic acid apart from maize, such as other cereals, legumes, oil seeds and nuts, with particular high phytate levels described for some nuts (6.3–9.4% dw) (Gupta et al., 2015); in maize, phytate contents up to 2.22 (% dw) have been reported (Schlemmer et al., 2009). Unlike other cereals, phytic acid in maize is mainly located in the germ (88%) (Feizollahi et al., 2021) that is usually removed during maize milling. Additionally, other processing methods used in maize grains (e.g. nixtamalisation,[26] fermentation) also decreases the levels of phytic acid increasing the bioavailability of different minerals and vitamins present in the grain (Suri and Tanumihardjo, 2016). During the assessment, it was also considered that the reported phytic acid levels in the GM-maize, although statistically significant different, barely differs from those in the conventional counterpart and in some of the selected non-GM reference varieties, and that similar and higher values are described in the literature for phytic acid in maize. Based on all this information, the GMO Panel concludes that the increased levels of phytic acid in the five-event stack GM maize as compared to the non-GM comparator do not represent a nutritional concern.

As compared to the non-GM comparator, a decrease of approximately 8% of vitamin B6[27] was observed in the grains from the five-event stack maize. Dietary reference values are set for vitamin B6; the most typical features of vitamin B6 deficiency, although rare, are hypochromic microcytic anaemia and neurological abnormalities (convulsive seizures, abnormal electroencephalograms) (EFSA NDA Panel, 2016b). Considering the magnitude of the decrease and that vitamin B6 is presented in many different foods (grains, pulses, nuts, seeds, potatoes and meat and meat products), this decrease is not considered of nutritional concern.

[25] Phytic acid is the principal storage compound of phosphorus in maize grains accounting for ~ 80% of the seeds total phosphorus (Raboy, 2009). The analysis of phosphorus in seeds was carried out after acid microwave digestion of the samples which releases the phosphorus bound to phytic acid allowing its analysis.

[26] Maize processing method which involves boiling the maize in water containing lime (calcium hydroxide) at a concentration range of 1–5% (Gómez et al., 1991).

[27] The term vitamin B6 is a generic descriptor for a group of 2-methyl,3-hydroxy,5-hydroxymethylpyridine derivatives. Vitamin B6 includes pyridoxine (PN), pyridoxal (PL) and pyridoxamine (PM), and their respective phosphorylated forms, pyridoxine 5′-phosphate (PNP), pyridoxal 5′-phosphate (PLP) and pyridoxamine 5′-phosphate (PMP). All these forms are present in food (EFSA NDA Panel, 2016b).

Behenic acid (C22:0) is a saturated fatty acid (FA) present at very low levels in maize (< 0.3% of total FA). Taking into account the relevance of this FA in the total FA content of maize grains, the increase of 3–5% as compared to the comparator is not considered nutritionally relevant.

3.4.3.6.2. Animal nutrition

The increase of ash content as compared to the conventional counterpart can be partially linked to the increase in phosphorus and potassium content, and does not pose an issue for animals.

Considering the very low levels of behenic acid in maize (< 0.3% total FA) and the observed increase as compared to the conventional counterpart, the nutritional impact in feeds is considered negligible.

Glycine is not an essential amino acid, although Wu et al. (2014) suggest that adequate provision of all amino acid is important to improve efficiency of animal production. Arginine, histidine and lysine are essential amino acids. Maize grains are not considered a major source of amino acids in animals and the increase of these amino acids is not a problem for animal nutrition.

Diets for animals are usually balanced for the content of major minerals, including phosphorus and potassium, and eventually supplemented when the amount provided by feed is not enough to satisfy nutritional requirements. The observed increase does not pose an issue for animals.

Pyridoxine is an important dietary vitamin especially for monogastric animals, and it is commonly added to the diet. The observed decrease in GM-treated maize compared to conventional counterpart does not pose an issue for animals.

Phytic acid is a source of phosphorus, but, especially for non-ruminant animals, is largely indigestible, and its availability can be increased by adding phytase in the diet. The observed increase is not a problem for animal nutrition.

3.4.3.7. Conclusions on the food/feed safety assessment

The Cry1A.105, Cry2Ab2, Cry1F, PAT, Vip3Aa20, PMI, CP4 EPSPS and AAD-1 proteins newly expressed in the five-event stack maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 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 five-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 five-event stack maize, or regarding the overall allergenicity of this five-event stack maize. Based on the outcome of the comparative assessment and the nutritional assessment, the GMO Panel concludes that the consumption of the five-event stack maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 does not represent any nutritional concern, in the context of the scope of this application.

3.4.4. Environmental risk assessment[28]

Considering the scope of application EFSA-GMO-NL-2018-151, which excludes cultivation, the environmental risk assessment (ERA) of the five-event stack maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 mainly takes into account: (1) the exposure of microorganisms to recombinant DNA in the gastrointestinal tract of animals fed GM material and of microorganisms present in environments exposed to faecal material of these animals (manure and faeces); and (2) the accidental release into the environment of viable five-event stack maize grains during transportation and/or processing (EFSA GMO Panel, 2010a).

3.4.4.1. 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. Survival is limited mainly by a combination of low competitiveness, absence of a dormancy phase and susceptibility to plant pathogens, herbivores and cold climate conditions (OECD, 2003), even though occasional feral GM maize plants may occur outside cultivation areas in the EU (e.g. Pascher, 2016). 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

[28] Dossier: Part II – Section 5; additional information: 11/2/2022; 8/6/2022.

(Palaudelmàs et al., 2009). Thus, the establishment and survival of feral and volunteer maize in the EU is currently limited and transient.

It is unlikely that the intended traits of the five-event stack maize will provide a selective advantage to maize plants, except when they are exposed to glyphosate-, glufosinate-, 2,4-D- and AAOP-containing herbicides or infested by insect pests that are susceptible to the Cry1A.105 and/or Cry2Ab2 and/or Cry1F and/or Vip3Aa proteins. However, if this was to occur this fitness advantage will not allow the GM plant to overcome other biological and abiotic factors (described above) limiting plant's persistence and invasiveness. Therefore, the presence of the intended traits will not affect the persistence and invasiveness of the GM plant.

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

3.4.4.2. Potential for gene transfer

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer (HGT) of DNA, or through vertical gene flow via cross-pollination from feral plants originating from spilled grains.

Plant-to-microorganism gene transfer

The probability and potential adverse effects of HGT of the recombinant DNA have been assessed in previous GMO Panel Scientific Opinions for the single events (see Table 1). This assessment included consideration of homology-based recombination processes, as well as non-homologous end joining and microhomology-mediated end joining. Possible fitness advantages that the bacteria in the receiving environments would gain from acquiring recombinant DNA were considered. No concern as a result of an unlikely, but theoretically possible, HGT of the recombinant genes to bacteria in the gut of domesticated animals and humans fed GM material or other receiving environments was identified.

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

The updated bioinformatics analyses of events MON 89034, 1507, MIR162, NK603 and DAS-40278-9 confirm the assessments provided in the context of previous Scientific Opinions (EFSA GMO Panel, 2019f, 2021a,b,c).

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

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

Plant-to-plant gene transfer

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

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

Maize is an annual predominantly cross-pollinating crop. Cross-fertilisation occurs mainly by wind (OECD, 2003). Vertical gene transfer from maize is limited to *Zea* species. Wild relatives of maize outside cultivation are not known/reported in Europe (Eastham and Sweet, 2002; OECD, 2003; EFSA, 2016, 2022; 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, 2022; 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, the likelihood/frequency of cross-pollination between occasional feral GM maize plants resulting from grain spillage, and weedy or cultivated *Zea* plants is considered extremely low (EFSA, 2016, 2022). 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-2018-151 into account (no cultivation), potential interactions of occasional feral five-event stack maize plants arising from grain import spills with the target organisms are not considered a relevant issue.

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

Given that environmental exposure of non-target organisms to spilled GM grains or occasional feral GM maize plants arising from spilled five-event stack maize grains is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions of five-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 and Vip proteins will not alter this conclusion.

3.4.4.5. Interactions with abiotic environment and biogeochemical cycles

Given that environmental exposure to spilled grains or occasional feral five-event stack maize plants arising from grain import spills is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions with the abiotic environment and biogeochemical cycles are not considered 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 five-event stack maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 would differ from conventional maize varieties in its ability to persist under European environmental conditions. Considering the scope of application EFSA-GMO-NL-2018-151, interactions of occasional feral five-event stack maize plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from five-event stack maize to bacteria does not indicate a safety concern. Therefore, considering the introduced traits, the outcome of the agronomic and phenotypic analysis, and the routes and levels of exposure, the GMO Panel concludes that five-event stack maize would not raise safety concerns in the event of accidental release of viable GM maize grains into the environment.

3.5. Risk assessment of the subcombinations[29]

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 16 subcombinations and no safety concerns were identified (see Table 2). Literature searches covering the 10 years before submission of the application and the period since the time of validity of the application revealed no new scientific information relevant to the risk assessment of these maize stacks. Consequently, the GMO Panel considers that its previous conclusions on these subcombinations remain valid.

3.5.2. Subcombinations not previously assessed

Nine of the 25 subcombinations included in the scope of this application have not been previously assessed by the GMO Panel (Table 9). In this case, following the strategy defined by the GMO Panel,[30] the risk assessment takes as its starting point the assessment of the single maize events, and uses the data generated for the five-event stack as well as all the additional data available on subcombinations previously assessed by the GMO Panel (Table 2) and the additional studies provided by the applicant (Appendix A).

[29] Additional information 22/2/2019.
[30] Annex 1 of the minutes: http://www.efsa.europa.eu/sites/default/files/event/170517-m.pdf

Table 9: Maize stacks not previously assessed and covered by the scope of application EFSA-GMO-NL-2018-151

Degree of stacking	Event
4-event stack	NK603 × MON 89034 × MIR162 × DAS-40278-9
	1507 × MON 89034 × MIR162 × DAS-40278-9
	1507 × NK603 × MIR162 × DAS-40278-9
	1507 × NK603 × MON 89034 × MIR162
3-event stack	MON 89034 × MIR162 × DAS-40278-9
	NK603 × MIR162 × DAS-40278-9
	1507 × MIR162 × DAS-40278-9
	1507 × MON 89034 × MIR162
2-event stack	MIR162 × DAS-40278-9

3.5.2.1. Stability of the events

The genetic stability of the inserted DNA over multiple generations in the five single maize events was demonstrated previously (see Table 2). Integrity of the events was demonstrated in the five-event stack maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 (Section 3.4.1.2) and the previously assessed maize subcombinations (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 8).

3.5.2.2. Expression of the events

The GMO Panel assessed whether any combination of the five events by conventional crossing could result in significant changes in expression levels of the newly expressed proteins, as this could indicate an unexpected interaction among 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 nine subcombinations compared with those in the single maize events. This assumption was confirmed by comparing the levels of the newly expressed proteins of each single maize event with those of the five-event stack maize. The levels were similar in the five-event stack maize and in the single events (Section 3.4.1.3 and Appendix B). Therefore, there was no indication of an interaction at protein expression level. This supports the conclusion that interactions affecting the expression levels of the newly expressed proteins are not expected in the nine subcombinations not previously assessed and included in the scope of application EFSA-GMO-NL-2018-151.

3.5.2.3. Potential functional interactions between the events

The GMO Panel assessed the potential for interactions among maize events in the nine subcombinations not previously assessed (Table 8), taking into consideration intended traits and unintended effects.

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

3.5.3. Conclusion

Since no new safety concerns were identified for the previously assessed subcombinations, the GMO Panel considers that its previous conclusions on these maize subcombinations remain valid. For the remaining nine subcombinations included in the scope of application EFSA-GMO-NL-2018-151, the GMO Panel assessed the possibility of interactions among the events and concluded that these combinations would not raise safety concerns. These subcombinations are therefore expected to be as safe as and nutritionally equivalent to the single maize events, the previously assessed subcombinations and the five-event stack maize.

3.6. Post-market monitoring

3.6.1. Post-market monitoring of GM food/feed

The GMO Panel concluded that the five-event stack maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9, as described in this application, does not raise any nutritional concern and is as safe as the comparator and the non-GM reference varieties tested (Section 3.4.3). Sixteen of the subcombinations have been previously assessed and no safety concerns were identified. The subcombinations not previously assessed and included in the scope of this application (nine) are expected to be as safe as the single maize events, the previously assessed maize subcombinations and the five-event stack maize (Section 3.5.2). Therefore, the GMO Panel considers that post-market monitoring of food and feed from the five-event stack maize and its subcombinations, as described in this application, is not necessary.

3.6.2. Post-market environmental monitoring

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

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

As the ERA did not identify potential adverse environmental effects from five-event stack maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for five-event stack maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 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 CropLife Europe 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 five-event stack maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

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

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

3.6.3. Conclusions on post-market monitoring

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

4. Overall conclusions

The GMO Panel was asked to carry out a scientific assessment of maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 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 five single maize events (MON 89034, 1507, MIR162, NK603 and DAS-40278-9) 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 in the five-event stack maize does not give rise to food/feed safety and nutritional concerns. The GMO Panel concludes

that the five-event stack maize, as described in this application, does not raise any nutritional concern and is as safe as its 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 five-event stack maize into the environment. Since no new data were identified on the 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 subcombinations included in the scope of application EFSA-GMO-NL-2018-151, no information has been provided. The GMO Panel assessed the possible interactions between the events in these subcombinations and concludes that these combinations of events MON 89034, 1507, MIR162, NK603 and DAS-40278-9 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 five-event stack maize.

Based on the relevant publications identified through the literature searches, the GMO Panel did not identify any safety issues pertaining to the intended uses of maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 and its subcombinations.

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 five-event stack maize and its subcombinations. Given the absence of safety and nutritional concerns for foods and feeds from the five-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 five-event stack maize and its subcombinations. In conclusion, the GMO Panel considers that maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 and its subcombinations, as described in this application, are as safe as the 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 (if appropriate)

- Letter from the Competent Authority of The Netherlands received on 31 May 2018 concerning a request for authorization of the placing on the market of genetically modified maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 submitted in accordance with Regulation (EC) No 1829/2003 by Dow AgroSciences Belgium B.V. on behalf of Dow AgroSciences LLC (EFSA-GMO-NL-2018-151; EFSA-Q-2018-00457).
- The application was made valid on 15 October 2018.
- Additional Information (1) was requested on 20 November 2018.
- Additional Information (1) was received on 22 February 2019.
- Additional Information (2) was requested on 12 December 2018.
- Additional Information (2) was received on 18 March 2019.
- Additional Information (3) was requested on 29 January 2019 (EURL).
- Additional Information (3) was received on 27 March 2019.
- Additional Information (4) was requested on 08 February 2019.
- Additional Information (4) was received on 25 March 2019.
- Additional Information (5) was requested on 11 March 2019.
- Additional Information (5) was received on 17 June 2019.
- Additional Information (6) was requested on 21 June 2019.
- Additional Information (6) was received on 11 November 2019.
- Additional Information (7) was requested on 09 December 2019.
- Additional Information (7) was received on 27 August 2021.
- Additional Information (8) was requested on 04 May 2020.
- Additional Information (8) was received on 16 July 2020.
- Additional Information (9) was requested on 20 October 2020.
- Additional Information (9) was received on 12 February 2021.
- Additional Information (10) was requested on 06 May 2021.
- Additional Information (10) was received on 09 July 2021.
- Additional Information (11) was requested on 08 October 2021.
- Additional Information (11) was received on 17 November 2021.
- Additional Information (12) was requested on 09 December 2021.

- Additional Information (12) was received on 11 February 2022 partial; 05 April 2022 partial; 12 May 2022 complete.
- Additional Information (13) was requested on 03 February 2022.
- Additional Information (13) was received on 02 March 2022.
- Additional Information (14) was requested on 04 March 2022.
- Additional Information (14) was received on 08 June 2022.
- Supplementary information was provided on voluntary basis on 27 November 2019; 16 April 2020 and 24 June 2021.

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Abbreviations

ADF	acid detergent fibre
bw	body weight
CaMV	cauliflower mosaic virus
CRM	comparative relative maturity
CTP	chloroplast transit peptide
DNA	deoxyribonucleic acid
dw	dry weight
ELISA	enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	environmental risk assessment
FMV	Figwort Mosaic Virus
GM	genetically modified
GMO	genetically modified organism
GMO Panel	EFSA Panel on Genetically Modified Organisms
HGT	horizontal gene transfer
hsp	heat shock proteins
LOD	limit of detection
MS	Member States
NDF	neutral detergent fibre
nos	nopaline synthase
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PAT	phosphinothricin-acetyl-transferase
PMEM	post-market environmental monitoring
UL	tolerable upper intake level
USA	United States of America
UTR	untranslated region

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 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 for humans, animal or the environment.

Study identification	Title
141211	(2015) Molecular Characterisation of the MON-89Ø34-3 × DAS-Ø15Ø7-1 × SYN-IR162-4 × MON-ØØ6Ø3-6 Maize Breeding Stack.
141098.B	(2015) Protein Expression of a Hybrid Maize Breeding Stack MON-89Ø34-3 × DAS-Ø15Ø7-1 × SYN-IR162-4 × MON-ØØ6Ø3-6 Containing Cry1A.105, Cry2Ab2, Cry1F, PAT, CP4 EPSPS, Vip3Aa20, and PMI Proteins.
141098.A	(2015) Nutrient Composition of a Maize hybrid breeding stack MON-89Ø34-3 × DAS-Ø15Ø7-1 × SYN-IR162-4 × MON-ØØ6Ø3-6 Maize According to EFSA Guidelines.
141098.C	(2015) Field Production and Agronomic Analysis of MON-89Ø34-3 × DAS-Ø15Ø7-1 × SYN-IR162-4 × MON-ØØ6Ø3-6 Maize According to EFSA Guidelines.
14050.4120	(2016) An 8-Week Dietary Tolerance Study of the Channel Catfish (*Ictalurus punctatus*) to Meal from MON-89Ø34-3 × DAS-Ø15Ø7-1 × SYN-IR162-4 × MON-ØØ6Ø3-6 Maize.
151077.H	(2017) Compositional and Agronomic Analysis of MON-89Ø34-3 × DAS-Ø15Ø7-1 × SYN-IR162-4 × MON-ØØ6Ø3-6 × DAS-4Ø278-9 Maize According to EFSA Guidelines

Appendix B – List of relevant publications identified by the applicant through systematic literature searches (1 January 2008–20 January 2022)

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Appendix C – Protein expression data

Mean, standard deviation and range of protein levels (ng/mg dry weight) from maize MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9 (not treated) and MON 89034, 1507, MIR162, NK603, DAS-40278-9 (not treated), from field trials performed across eight locations in Argentina in 2015-2016 (n = 32).

Protein	Event(s)	Leaf (V2-V4)	Leaf (V9)	Leaf (R1)	Pollen (R1)	Root (R1)	Grain (R6)	Forage (R5)
Cry1A.105	**MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9**	24[a] ± 5.9[b] (12–46)[c]	19 ± 5.6 (9.8–33)	13 ± 3.9 (6.1–23)	3.0 ± 0.50 (1.7–3.9)	16 ± 5.5 (8.4–30)	0.46 ± 0.14 (0.25–0.87)	5.2 ± 1.7 (2.7–10)
	MON 89034	24 ± 5.3 (15–34)	12 ± 4.0 (6.9–21)	22 ± 6.3 (11–41)	2.5 ± 0.51 (1.4–3.7)	17 ± 4.4 (9.8–27)	0.39 ± 0.08 (0.20–0.51)	6.0 ± 1.6 (2.4–9.9)
Cry2Ab2	**MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9**	250 ± 49 (150–340)	240 ± 80 (99–410)	210 ± 34 (140–290)	4.0 ± 0.72 (2.1–5.4)	110 ± 26 (68–160)	9.2 ± 3.3 (4.1–19)	78 ± 32 (22–200)
	MON 89034	240 ± 63 (120–380)	230 ± 77 (120–400)	210 ± 36 (140–290)	2.5 ± 0.57 (1.5–3.7)	100 ± 24 (63–150)	7.2 ± 1.8 (3.0–11)	130 ± 53 (43–220)
Cry1F	**MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9**	22 ± 5.5 (15–34)	12 ± 2.9 (7.4–17)	31 ± 11 (17–52)	18 ± 1.9 (13–21)	9.2 ± 1.8 (6.0–13)	3.9 ± 1.1 (1.8–7.4)	4.9 ± 1.4 (2.7–8)
	1507	31 ± 7.1 (22–49)	12 ± 4.0 (4.8–21)	25 ± 8.5 (14–37)	25 ± 5.1 (14–32)	5.2 ± 1.3 (2.9–8.2)	3.2 ± 0.62 (1.8–4.9)	4.6 ± 1.6 (2.7 ± 11)
PAT	**MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9**	3.8 ± 0.95 (2.3–6.0)	4.0 ± 0.77 (2.8–5.8)	4.3 ± 1.5 (1.7–8.1)	< LOD[e]	0.18 ± 0.050 (0.10–0.30)	< LOD[e]	0.023 ± 0.046 (< LOD-0.13)
	1507	4.2 ± 0.79 (3.0–6.1)	4.4 ± 0.60 (3.2–5.9)	3.3 ± 1.5 (1.4–7.4)	< LOD[e]	0.21 ± 0.061 (0.12–0.34)	< LOD[e]	0.080 ± 0.069 (< LOD-0.21)
Vip3Aa20	**MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9**	180 ± 60 (110–340)	54 ± 12 (32–79)	170 ± 44 (100–250)	52 ± 6.3 (45–68)	49 ± 13 (27–78)	50 ± 20 (29–140)	61 ± 17 (35–110)
	MIR162	160 ± 38 (100–240)	64 ± 17 (36–110)	150 ± 42 (83–240)	49 ± 5.6 (40–63)	48 ± 12 (25–75)	45 ± 16 (24–89)	64 ± 22 (44–140)
PMI	**MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9**	8.7 ± 2.1 (4.9–13)	5.4 ± 1.3 (3.2–8.6)	13 ± 2.6 (8.0–19)	4.3 ± 0.54 (3.4–5.4)	2.3 ± 0.63 (1.4–3.8)	2.6 ± 0.85 (1.4–5.8)	3.1 ± 0.84 (1.5–5.1)
	MIR162	9.1 ± 1.2 (7.1–11)	6.3 ± 1.3 (4.5–8.9)	14 ± 2.5 (9.3–18)	4.3 ± 0.52 (3.3–5.5)	2.1 ± 0.58 (0.96–3.5)	2.2 ± 0.60 (1.3–3.7)	2.9 ± 0.64 (1.8–4.3)

Protein	Event(s)	Leaf (V2-V4)	Leaf (V9)	Leaf (R1)	Pollen (R1)	Root (R1)	Grain (R6)	Forage (R5)
CP4 EPSPS[(d)]	**MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9**	250 ± 40 (170–350)	220 ± 34 (140–300)	290 ± 35 (210–360)	290 ± 40 (210–360)	33 ± 9.4 (19–57)	13 ± 4.2 (6.0–29)	28 ± 10 (11–55)
	NK603	230 ± 38 (160–330)	200 ± 31 (140–290)	260 ± 37 (190–340)	250 ± 61 (160–400)	27 ± 7.8 (15–47)	11 ± 2.5 (4.6–18)	25 ± 9.9 (7.9–47)
AAD-1	**MON 89034 × 1507 × MIR162 × NK603 × DAS-40278-9**	12 ± 4.2 (5.9–21)	5.6–1.5 (2.6–9.7)	12 ± 2.3 (6.7–16)	140 ± 18 (99–170)	5.6 ± 2.4 (3.0–13)	3.6 ± 1.0 (2.3–6.8)	4.0 ± 1.4 (2.8–8.2)
	DAS-40278-9	13 ± 6.0 (6.3–27)	6.4 ± 1.5 (3.7–9.3)	11 ± 2.3 (7.1–16)	110 ± 21 (70–150)	6.0 ± 2.4 (3.0–13)	3.5 ± 0.89 (2.1–5.8)	3.5 ± 0.95 (2.3–6.4)

(a): Mean.
(b): Standard deviation.
(c): Range.
(d): CP4 EPSPS levels are a sum of two protein variants CP4 EPSPS and CP4 EPSPS L214P, both expressed in maize NK603.
(e): all samples resulted below the limit of detection (LOD = 0.025 ng/mg).

Appendix D – Animal dietary exposure

Table D.1: Dietary exposure to Cry1A.105, Cry2Ab2, Cry1F, PAT, Vip3Aa20, PMI, CP4 EPSPS, and AAD-1 proteins (mg/kg bw per day) in selected animals, based on the consumption of maize grains and forage

Cry1A.105	BW (kg)	TDI feed (kg DM/animal)	IR (%) grains	IR (%) forage	Grain (G)	Forage (F)	G + F
chicken for fattening	2	0.158	70	NA	0.025	NA	NA
laying hen	2	0.106	70	10	0.017	0.028	0.045
turkey for fattening	3	0.176	50	NA	0.014	NA	NA
pig for fattening	60	2.20	70	NA	0.012	NA	NA
sow lactating	175	5.28	70	20	0.0097	0.031	0.041
cattle for fattening[(a)]	400	8	80	80	0.0074	0.083	0.091
dairy cow	650	20	30	60	0.0043	0.097	0.10
sheep/goat	60	1.2	30	NA	0.0028	NA	NA
salmon[(b)]	0.12	0.0021	10	NA	0.00083	NA	NA
dog	15	0.25	45	NA	0.0035	NA	NA
cat	3	0.06	25	NA	0.0023	NA	NA

Cry2Ab2	BW (kg)	TDI feed (kg DM/animal)	IR (%) grains	IR (%) forage	Grain (G)	Forage (F)	G + F
chicken for fattening	2	0.158	70	NA	0.47	NA	NA
laying hen	2	0.106	70	10	0.32	0.45	0.76
turkey for fattening	3	0.176	50	NA	0.25	NA	NA
pig for fattening	60	2.20	70	NA	0.22	NA	NA
sow lactating	175	5.28	70	20	0.18	0.50	0.68
cattle for fattening[(a)]	400	8	80	80	0.14	1.34	1.48
dairy cow	650	20	30	60	0.079	1.56	1.64
sheep/goat	60	1.2	30	NA	0.051	NA	NA
salmon[(b)]	0.12	0.0021	10	NA	0.015	NA	NA
dog	15	0.25	45	NA	0.065	NA	NA
cat	3	0.06	25	NA	0.043	NA	NA

Cry1F	BW (kg)	TDI feed (kg DM/animal)	IR (%) grains	IR (%) forage	Grain (G)	Forage (F)	G + F
chicken for fattening	2	0.158	70	NA	0.23	NA	NA
laying hen	2	0.106	70	10	0.15	0.026	0.18
turkey for fattening	3	0.176	50	NA	0.12	NA	NA
pig for fattening	60	2.20	70	NA	0.11	NA	NA
sow lactating	175	5.28	70	20	0.086	0.029	0.12
cattle for fattening[(a)]	400	8	80	80	0.066	0.078	0.14
dairy cow	650	20	30	60	0.038	0.091	0.13
sheep/goat	60	1.2	30	NA	0.025	NA	NA

Cry1F	BW (kg)	TDI feed (kg DM/animal)	IR (%) grains	IR (%) forage	Grain (G)	Forage (F)	G + F
salmon[(b)]	0.12	0.0021	10	NA	0.0074	NA	NA
dog	15	0.25	45	NA	0.031	NA	NA
cat	3	0.06	25	NA	0.021	NA	NA

PAT	BW (kg)	TDI feed (kg DM/ animal)	IR (%) grains	IR (%) forage	Grain (G)	Forage (F)	G + F
chicken for fattening	2	0.158	70	NA	0.0014	NA	NA
laying hen	2	0.106	70	10	0.00093	0.00013	0.0010
turkey for fattening	3	0.176	50	NA	0.00074	NA	NA
pig for fattening	60	2.20	70	NA	0.00065	NA	NA
sow lactating	175	5.28	70	20	0.00053	0.00015	0.00068
cattle for fattening[(a)]	400	8	80	80	0.00040	0.0004	0.0008
dairy cow	650	20	30	60	0.00023	0.00046	0.00069
sheep/goat	60	1.2	30	NA	0.00015	NA	NA
salmon[(b)]	0.12	0.0021	10	NA	0.000045	NA	NA
dog	15	0.25	45	NA	0.00019	NA	NA
cat	3	0.06	25	NA	0.00013	NA	NA

Vip3Aa20	BW (kg)	TDI feed (kg DM/animal)	IR (%) grains	IR (%) forage	Grain (G)	Forage (F)	G + F
chicken for fattening	2	0.158	70	NA	2.54	NA	NA
laying hen	2	0.106	70	10	1.71	0.32	2.02
turkey for fattening	3	0.176	50	NA	1.36	NA	NA
pig for fattening	60	2.20	70	NA	1.19	NA	NA
sow lactating	175	5.28	70	20	0.97	0.36	1.33
cattle for fattening[(a)]	400	8	80	80	0.74	0.96	1.70
dairy cow	650	20	30	60	0.43	1.12	1.54
sheep/goat	60	1.2	30	NA	0.28	NA	NA
salmon[(b)]	0.12	0.0021	10	NA	0.083	NA	NA
dog	15	0.25	45	NA	0.35	NA	NA
cat	3	0.06	25	NA	0.23	NA	NA

PMI	BW (kg)	TDI feed (kg DM/animal)	IR (%) grains	IR (%) forage	Grain (G)	Forage (F)	G + F
chicken for fattening	2	0.158	70	NA	0.14	NA	NA
laying hen	2	0.106	70	10	0.093	0.016	0.11
turkey for fattening	3	0.176	50	NA	0.074	NA	NA
pig for fattening	60	2.20	70	NA	0.065	NA	NA

PMI	BW (kg)	TDI feed (kg DM/animal)	IR (%) grains	IR (%) forage	Grain (G)	Forage (F)	G + F
sow lactating	175	5.28	70	20	0.053	0.019	0.071
cattle for fattening[(a)]	400	8	80	80	0.040	0.050	0.090
dairy cow	650	20	30	60	0.023	0.058	0.081
sheep/goat	60	1.2	30	NA	0.015	NA	NA
salmon[(b)]	0.12	0.0021	10	NA	0.0045	NA	NA
dog	15	0.25	45	NA	0.019	NA	NA
cat	3	0.06	25	NA	0.013	NA	NA

CP4 EPSPS	BW (kg)	TDI feed (kg DM/animal)	IR (%) grains	IR (%) forage	Grain (G)	Forage (F)	G + F
chicken for fattening	2	0.158	70	NA	1.00	NA	NA
laying hen	2	0.106	70	10	0.67	0.16	0.83
turkey for fattening	3	0.176	50	NA	0.53	NA	NA
pig for fattening	60	2.20	70	NA	0.47	NA	NA
sow lactating	175	5.28	70	20	0.38	0.19	0.56
cattle for fattening[(a)]	400	8	80	80	0.29	0.50	0.78
dairy cow	650	20	30	60	0.17	0.58	0.74
sheep/goat	60	1.2	30	NA	0.11	NA	NA
salmon[(b)]	0.12	0.0021	10	NA	0.032	NA	NA
dog	15	0.25	45	NA	0.14	NA	NA
cat	3	0.06	25	NA	0.090	NA	NA

AAD-1	BW (kg)	TDI feed (kg DM/animal)	IR (%) grains[(c)]	IR (%) forage	Grain (G)	Forage (F)	G + F
chicken for fattening	2	0.158	70	NA	0.19	NA	NA
laying hen	2	0.106	70	10	0.13	0.022	0.15
turkey for fattening	3	0.176	50	NA	0.10	NA	NA
pig for fattening	60	2.20	70	NA	0.088	NA	NA
sow lactating	175	5.28	70	20	0.071	0.025	0.096
cattle for fattening[(a)]	400	8	80	80	0.054	0.066	0.12
dairy cow	650	20	30	60	0.032	0.076	0.11
sheep/goat	60	1.2	30	NA	0.020	NA	NA
salmon[(b)]	0.12	0.0021	10	NA	0.0061	NA	NA
dog	15	0.25	45	NA	0.026	NA	NA
cat	3	0.06	25	NA	0.017	NA	NA

NA indicates that a forage inclusion rate was not provided in the reference and therefore no exposure calculations were done.
(a): The inclusion rate for beef cattle would be 160% of the diet, resulting the DDE to each protein an overestimation.
(b): The dietary exposure in salmon was based on the levels of the newly expressed proteins in maize grains, without applying an adjusting factor. The GMO Panel considers that crude protein in maize gluten meal increases by a factor of 7.1 after processing, based on the protein content of gluten meal relative to maize grains (OECD, 2002), assuming that no protein is lost during the processing. Therefore, the above-reported values for the estimation of dietary exposure to newly expressed proteins in salmon should be adjusted accordingly.

(c): The inclusion rates (IR) are derived from OECD (2013) for livestock animals; FAO (2017) for salmon and additional information for cat and dog.[31]

[31] Additional information: 11/2/2022 (Annex 3: Communication from Purina (LabDiet) to Corteva Agriscience (Pioneer) regarding companion animal dietary ingredient incorporation rates).