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Assessment of genetically modified maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 and 30 subcombinations, for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-DE-2018-149)

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Abstract

Genetically modified maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 was developed by crossing to combine six single events: Bt11, MIR162, MIR604, MON 89034, 5307 and GA21, the GMO Panel previously assessed the 6 single maize events and 27 out of the 56 possible 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 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 six-event stack maize does not give rise to food and feed safety and nutritional concerns. The GMO Panel concludes that six-event stack maize, as described in this application, is as safe as the conventional counterpart and non-GM maize varieties tested, and no post-market monitoring of food/feed is considered necessary. In the case of accidental release of viable six-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 29 of the maize subcombinations not previously assessed and covered by the scope of this application and concludes that these are expected to be as safe as the single events, the previously assessed subcombinations and the six-event stack maize. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21. The GMO Panel concludes that six-event stack maize and the 30 subcombinations covered by the scope of the application are as safe as its conventional counterpart and the tested non-GM maize varieties with respect to potential effects on human and animal health and the environment.

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

Requestor: Competent Authority of Germany

Question number: EFSA-Q-2018-00292

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Summary

Following the submission of application EFSA-GMO-DE-2018-149 under Regulation (EC) No 1829/2003 from Syngenta Crop Protection NV/SA (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.) Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 (referred to hereafter as 'six-event stack maize') and 30 subcombinations independently of their origin, according to Regulation (EU) No 503/2013 (referred to hereafter as 'subcombinations'). The scope of application EFSA-GMO-DE-2018-149 is for import, processing and food and feed uses within the European Union (EU) of maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 and does not include cultivation in the EU. The term 'subcombination' refers to any combination of up to five of the events present in the six-event stack maize. The safety of subcombinations occurring as segregating progeny in the harvested grains of maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 is evaluated in the context of the assessment of the six-event stack maize. The safety of subcombinations that have either been or could be produced by crossing through targeted breeding approaches, and which can be bred, produced and marketed independently of the six-event stack maize, are risk assessed separately in the present scientific opinion.

The six-event stack maize was produced by crossing to combine six single maize events:

Bt11, expressing the Cry1Ab protein for protection against certain lepidopteran pests and the phosphinothricin acetyl transferase (PAT) protein for tolerance to glufosinate-ammonium-containing herbicides;

MIR162, expressing the Vip3Aa20 protein against certain lepidopteran pests and the phosphomannose isomerase (PMI) protein used as a selectable marker;

MIR604, expressing a modified Cry3A (mCry3A) protein against certain coleopteran pests and the PMI protein used as a selectable marker;

MON 89034 expressing Cry1A.105 and Cry2Ab2 proteins for protection against certain lepidopteran pests;

5307, expressing the eCry3.1Ab protein against certain coleopteran pests and the PMI protein used as a selectable marker;

GA21, expressing the 5-enolpyruvylshikimate-3-phosphate synthase enzyme (mEPSPS) protein for tolerance to glyphosate-containing herbicides.

The GMO Panel evaluated the six-event stack maize and 30 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-DE-2018-149, 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-DE-2018-149, previous assessments of the six single events (Bt11, MIR162, MIR604, MON 89034, 5307 and GA21), and 27 of the subcombinations provided a basis for the assessment of the six-event stack maize and 30 subcombinations. No safety concerns were identified by the GMO Panel in the previous assessments. No safety issue concerning the six single maize events was identified by the updated bioinformatic analyses, or 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. The GMO Panel noted that the applicant did not inform EFSA of the existence of a patent related to maize MIR162. The patent was independently identified in a public consultation and assessed by the GMO Panel; no safety concerns were identified. The GMO Panel considers that the applicants should guarantee the timely delivery of potentially relevant scientific information to EFSA to assist in the processing of applications.

For the six-event stack maize, the risk assessment included the molecular characterisation of the inserted DNA and analysis of protein expression. An evaluation of the comparative analysis of agronomic, phenotypic and compositional characteristics was carried out and the safety of the newly expressed proteins 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 Bt11, MIR162, MIR604, MON 89034, 5307 and GA21 combined in the six-event stack maize

have retained their integrity. Protein expression analysis showed that the levels of the newly expressed proteins are similar in the six-event stack maize and in the single events.

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 are appropriate to support the comparative analysis. The comparative analysis of agronomic and phenotypic characteristics and grain and forage composition identified no differences between the six-event stack maize and the non-GM comparator (referred to hereafter as comparator) that required further assessment except for the changes in NDF, stearic acid (C18:0), ferulic acid and *p*-coumaric acid. These changes were further assessed for food/feed safety 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 six-event stack maize does not give rise to food and feed safety and nutritional concerns. The GMO Panel concludes that the six-event stack maize, 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 the six-event stack maize 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-DE-2018-149, experimental data were provided for maize Bt11 × MIR162 × MON 89034 × GA21 and maize Bt11 × MIR162 × MON 89034 (see Appendix A). 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 six-event stack maize.

Given the absence of safety concerns for foods and feeds from the six-event stack maize and 30 subcombinations, the GMO Panel considers that post-market monitoring of these products is not necessary. The PMEM plan and reporting intervals are in line with the intended uses of the six-event stack maize and 30 subcombinations.

The GMO Panel concludes that the six-event stack maize and 30 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-DE-2018-149 is for food and feed uses, import and processing of the genetically modified (GM) herbicide-tolerant and insect-resistant maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 and 30 subcombinations independently of their origin and does not include cultivation in the European Union (EU).

1.1. Background

On 13 April 2018, the European Food Safety Authority (EFSA) received from the Competent Authority of Germany application EFSA-GMO-DE-2018-149 for authorisation of maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 (Unique Identifier SYN-BTØ11-1 × SYN-IR6Ø4–5 × MON-ØØØ21-9 × MON-89Ø34-3 × SYN-IR162-4 × SYN-Ø53Ø7–1), submitted by Syngenta Crop Protection NV/SA (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003[1]. Following receipt of application EFSA-GMO-DE-2018-149, EFSA informed EU Member States (MS) and the European Commission (EC), and made the application available to them. Simultaneously, EFSA published 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 6 July 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 six months to issue a scientific opinion on application EFSA-GMO-DE-2018-149. 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 three months to make their opinion known on application EFSA-GMO-DE-2018-149 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 Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 in the context of its scope as defined in application EFSA-GMO-DE-2018-149.

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. 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.[5]

2. Data and Methodologies

2.1. Data

The GMO Panel based its scientific assessment of six-event stack maize on the valid application EFSA-GMO-DE-2018-149, additional information provided by the applicant during the risk assessment, relevant scientific comments submitted by EU MS and relevant peer-reviewed scientific publications. As

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.
[2] Available online: https://open.efsa.europa.eu/study-inventory/EFSA-Q-2018-00292
[3] Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. OJ L157, 8.6.2013, p. 1–48.
[4] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 12.3.2001, p. 1–38.
[5] These particulars are available online: https://open.efsa.europa.eu/study-inventory/EFSA-Q-2018-00292

part of this comprehensive information package, the GMO Panel received additional unpublished studies submitted by the applicant 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, 2015a, 2017a, 2021a; EFSA Scientific Committee, 2011) and explanatory notes and statements (i.e. EFSA GMO Panel, 2010b; EFSA, 2010, 2014, 2017, 2019a,b) for the risk assessment of GM plants.

For this application, in the context of the contracts, OC/EFSA/GMO/2018/02 – lot 1 and 2 - and EOI/EFSA/SCIENCE/2020/01 – CT02GMO the contractors performed preparatory work for the evaluation of the methods applied for the statistical analysis of agronomic, phenotypic and composition (lot 1), and of the statistical analysis and overall design (lot 2) of 90-day toxicity studies on maize 5307, Bt11 and MIR604.

3. Assessment

3.1. Introduction

Application EFSA-GMO-DE-2018-149 covers the maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 and 30 subcombinations, out of the possible 56, independently of their origin (see Table 1).

Table 1: The six-event stack maize event and the 30 subcombinations covered by the scope of application EFSA-GMO-DE-2018-149

Degree of stacking	Events	Unique identifiers
Six-event stack	Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21	SYN-BTØ11-1 × SYN-IR6Ø4–5 × MON-ØØØ21-9 × MON-89Ø34-3 × SYN-IR162-4 × SYN-Ø53Ø7–1
Five-event stack	MIR604 × GA21 × MON 89034 × MIR162 × 5307	SYN-IR6Ø4–5 × MON-ØØØ21-9 × MON-89Ø34-3 × SYN-IR162-4 × SYN-Ø53Ø7–1
	Bt11 × GA21 × MON 89034 × MIR162 × 5307	SYN-BTØ11-1 × MON-ØØØ21-9 × MON-89Ø34-3 × SYN-IR162-4 × SYN-Ø53Ø7–1
	Bt11 × MIR604 × MON 89034 × MIR162 × 5307	SYN-BTØ11-1 × SYN-IR6Ø4–5 × MON-89Ø34-3 × SYN-IR162-4 × SYN-Ø53Ø7–1
	Bt11 × MIR604 × GA21 × MON 89034 × 5307	SYN-BTØ11-1 × SYN-IR6Ø4–5 × MON-ØØØ21-9 × MON-89Ø34-3 × SYN-Ø53Ø7–1
	Bt11 × MIR604 × GA21 × MON 89034 × MIR162	SYN-BTØ11-1 × SYN-IR6Ø4–5 × MON-ØØØ21-9 × MON-89Ø34-3 × SYN-IR162-4
Four-event stack	GA21 × MON 89034 × MIR162 × 5307	MON-ØØØ21-9 × MON-89Ø34-3 × SYN-IR162-4 × SYN-Ø53Ø7–1
	MIR604 × MON 89034 × MIR162 × 5307	SYN-IR6Ø4–5 × MON-89Ø34-3 × SYN-IR162-4 × SYN-Ø53Ø7–1
	MIR604 × GA21 × MON 89034 × 5307	SYN-IR6Ø4–5 × MON-ØØØ21-9 × MON-89Ø34-3 × SYN-Ø53Ø7–1
	MIR604 × GA21 × MON 89034 × MIR162	SYN-IR6Ø4–5 × MON-ØØØ21-9 × MON-89Ø34-3 × SYN-IR162-4
	Bt11 × MON 89034 × MIR162 × 5307	SYN-BTØ11-1 × MON-89Ø34-3 × SYN-IR162-4 × SYN-Ø53Ø7–1
	Bt11 × GA21 × MON 89034 × 5307	SYN-BTØ11-1 × MON-ØØØ21-9 × MON-89Ø34-3 × SYN-Ø53Ø7–1
	Bt11 × GA21 × MON 89034 × MIR162	SYN-BTØ11-1 × MON-ØØØ21-9 × MON-89Ø34-3 × SYN-IR162-4
	Bt11 × MIR604 × MON 89034 × 5307	SYN-BTØ11-1 × SYN-IR6Ø4–5 × MON-89Ø34-3 × SYN-Ø53Ø7–1

Degree of stacking	Events	Unique identifiers
	Bt11 × MIR604 × MON 89034 × MIR162	SYN-BTØ11-1 × SYN-IR6Ø4–5 × MON-89Ø34-3 × SYN-IR162-4
	Bt11 × MIR604 × GA21 × MON 89034	SYN-BTØ11-1 × SYN-IR6Ø4–5 × MON-ØØØ21-9 × MON-89Ø34-3
Three-event stack	MON 89034 × MIR162 × 5307	MON-89Ø34-3 × SYN-IR162-4 × SYN-Ø53Ø7–1
	GA21 × MON 89034 × 5307	MON-ØØØ21-9 × MON-89Ø34-3 × SYN-Ø53Ø7–1
	GA21 × MON 89034 × MIR162	MON-ØØØ21-9 × MON-89Ø34-3 × SYN-IR162-4
	MIR604 × MON 89034 × 5307	SYN-IR6Ø4–5 × MON-89Ø34-3 × SYN-Ø53Ø7–1
	MIR604 × MON 89034 × MIR162	SYN-IR6Ø4–5 × MON-89Ø34-3 × SYN-IR162-4
	MIR604 × GA21 × MON 89034	SYN-IR6Ø4–5 × MON-ØØØ21-9 × MON-89Ø34-3
	Bt11 × MON 89034 × 5307	SYN-BTØ11-1 × MON-89Ø34-3 × SYN-Ø53Ø7–1
	Bt11 × MON 89034 × MIR162	SYN-BTØ11-1 × MON-89Ø34-3 × SYN-IR162-4
	Bt11 × GA21 × MON 89034	SYN-BTØ11-1 × MON-ØØØ21-9 × MON-89Ø34-3
	Bt11 × MIR604 × MON 89034	SYN-BTØ11-1 × SYN-IR6Ø4–5 × MON-89Ø34-3
Two-event stack	MON 89034 × 5307	MON-89Ø34-3 × SYN-Ø53Ø7–1
	MON 89034 × MIR162	MON-89Ø34-3 × SYN-IR162-4
	GA21 × MON 89034	MON-ØØØ21-9 × MON-89Ø34-3
	MIR604 × MON 89034	SYN-IR6Ø4–5 × MON-89Ø34-3
	Bt11 × MON 89034	SYN-BTØ11-1 × MON-89Ø34-3

The term 'subcombination' refers to any combination of up to five of the maize events Bt11, MIR162, MIR604, MON 89034, 5307 and GA21.

The safety of subcombinations occurring as segregating progeny in harvested grains of the six-event stack maize is evaluated in the context of the assessment of the six-event stack maize in Section 3.4 of the present scientific opinion.

The assessment of subcombinations also covers combinations that have either been or could be produced by conventional crossing through targeted breeding approaches[6] (EFSA GMO Panel, 2011a). These are maize stacks that can be bred, produced and marketed independently of the six-event stack maize. These subcombinations are assessed in Section 3.5 of this scientific opinion.

The six-event stack maize was produced by crossing to combine six single maize events: Bt11 (expressing Cry1A and PAT), MIR162 (expressing Vip3Aa20 and PMI), MIR604 (expressing mCry3A and PMI), MON 89034 (expressing Cry1A.105 and Cry2Ab2), 5307 (eCry3.1Ab and PMI) and GA21 (expressing mEPSPS) to confer resistance to certain lepidopteran (Bt11, MIR162, MON 89034) and coleopteran (MIR604, 5307) pests and tolerance to glufosinate-ammonium- and glyphosate-based herbicides (Bt11, GA21).

All six single events, 11 two-event stacks, 10 three-event stacks, 5 four-event stacks and 1 five-event stack were assessed previously (see Table 2) and no safety concerns for human and animal health or environmental safety were identified.

Table 2: Single maize events and the 27 subcombinations of maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 previously assessed by the GMO Panel

Events	Application or mandate	Reference
Bt11	C/F/96/05.10	EFSA (2005)
	RX-Bt11	EFSA (2009a)
	RX-016	EFSA GMO Panel (2021)
MIR604	AP 11	EFSA (2009b)
	RX-013	EFSA GMO Panel (2019a)
GA21	AP 19	EFSA (2007)

[6] The two subcombinations Bt11 × MIR162 × MON 89034 and Bt11 × MIR162 × MON 89034 × GA21 are produced by targeted breeding approaches and protein expression data were provided (see Appendix A).

Events	Application or mandate	Reference
	AP 60	EFSA GMO Panel (2011c)
	RX-GA21	EFSA (2007)
	RX-005	EFSA GMO Panel (2017b)
MON 89034	AP 37	EFSA (2008)
	RX-015	EFSA GMO Panel (2019b)
MIR162	AP 82	EFSA GMO Panel (2012)
	RX-025	EFSA GMO Panel (2022a)
	M-2022-00202	EFSA GMO Panel (2023)
5307	AP 95	EFSA GMO Panel (2015b)
	M-2017-0011	EFSA GMO Panel (2018)
Bt11 × MIR604	AP 50	EFSA GMO Panel (2010c)
	AP 66	EFSA GMO Panel (2015c)
	AP 103	EFSA GMO Panel (2019c)
Bt11 × GA21	AP 49	EFSA GMO Panel (2009)
	AP 66	EFSA GMO Panel (2015c)
	AP 103	EFSA GMO Panel (2019c)
Bt11 × MIR162	AP 66	EFSA GMO Panel (2015c)
	M-2016-0248	EFSA GMO Panel (2017c)
	AP 103	EFSA GMO Panel (2019c)
	M-2022-00202	EFSA GMO Panel (2023)
Bt11 × 5307	AP 103	EFSA GMO Panel (2019c)
MIR604 × GA21	AP 48	EFSA GMO Panel (2010d)
	AP 66	EFSA GMO Panel (2015c)
	AP 103	EFSA GMO Panel (2019c)
MIR604 × MIR162	AP 66	EFSA GMO Panel (2015c)
	AP 103	EFSA GMO Panel (2019c)
	M-2022-00202	EFSA GMO Panel (2023)
MIR604 × 5307	AP 103	EFSA GMO Panel (2019c)
GA21 × MIR162	AP 66	EFSA GMO Panel (2015c)
	AP 103	EFSA GMO Panel (2019c)
	M-2022-00202	EFSA GMO Panel (2023)
GA21 × 5307	AP 103	EFSA GMO Panel (2019c)
MON 89034 × MIR162[(a)]	AP 131	EFSA GMO Panel (2019d)
	AP 134	EFSA GMO Panel (2019e)
	AP 144	EFSA GMO Panel (2019f)
	AP 151	EFSA GMO Panel (2022b)
	M-2022-00202	EFSA GMO Panel (2023)
MIR162 × 5307	AP 103	EFSA GMO Panel (2019c)
	M-2022-00202	EFSA GMO Panel (2023)
Bt11 × MIR604 × GA21	AP 56	EFSA GMO Panel (2010e)
	AP 66	EFSA GMO Panel (2015c)
	AP 103	EFSA GMO Panel (2019c)
Bt11 × MIR604 × MIR162	AP 66	EFSA GMO Panel (2015c)
	AP 103	EFSA GMO Panel (2019c)
	M-2022-00202	EFSA GMO Panel (2023)
Bt11 × MIR604 × 5307	AP 103	EFSA GMO Panel (2019c)
Bt11 × GA21 × MIR162	AP 66	EFSA GMO Panel (2015c)
	AP 103	EFSA GMO Panel (2019c)
	M-2022-00202	EFSA GMO Panel (2023)
Bt11 × GA21 × 5307	AP 103	EFSA GMO Panel (2019c)

Events	Application or mandate	Reference
Bt11 × MIR162 × 5307	AP 103	EFSA GMO Panel (2019c)
	M-2022-00202	EFSA GMO Panel (2023)
MIR604 × GA21 × MIR162	AP 66	EFSA GMO Panel (2015c)
	AP 103	EFSA GMO Panel (2019c)
	M-2022-00202	EFSA GMO Panel (2023)
MIR604 × GA21 × 5307	AP 103	EFSA GMO Panel (2019c)
MIR604 × MIR162 × 5307	AP 103	EFSA GMO Panel (2019c)
	M-2022-00202	EFSA GMO Panel (2023)
GA21 × MIR162 × 5307	AP 103	EFSA GMO Panel (2019c)
	M-2022-00202	EFSA GMO Panel (2023)
Bt11 × MIR604 × GA21 × MIR162	AP 66	EFSA GMO Panel (2015c)
	AP 103	EFSA GMO Panel (2019c)
	M-2022-00202	EFSA GMO Panel et al. (2023)
Bt11 × MIR604 × GA21 × 5307	AP 103	EFSA GMO Panel (2019c)
Bt11 × MIR604 × MIR162 × 5307	AP 103	EFSA GMO Panel (2019c)
	M-2022-00202	EFSA GMO Panel (2023)
Bt11 × GA21 × MIR162 × 5307	AP 103	EFSA GMO Panel (2019c)
	M-2022-00202	EFSA GMO Panel (2023)
MIR604 × GA21 × MIR162 × 5307	AP 103	EFSA GMO Panel (2019c)
	M-2022-00202	EFSA GMO Panel (2023)
Bt11 × MIR604 × GA21 × MIR162 × 5307	AP 103	EFSA GMO Panel (2019c)
	M-2022-00202	EFSA GMO Panel (2023)

(a): Maize MON 89034 × MIR162 is part of the 30 subcombinations covered by the scope of this application. At the time of submission, maize MON 89034 × MIR162 was not yet risk assessed by the GMO Panel. Currently this double stack has been risk assessed in the frame of several higher stacks.

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 concerning the six single events has been reported by the applicant.

The GMO Panel noted, however, that the text of a recent European patent, property of the applicant, points to a potential link between event MIR162 and altered male fertility. This information is novel and potentially relevant to the safety of maize MIR162. The applicant did not inform EFSA of the existence of this patent. The patent was independently identified in a public consultation and the potential related issues have been assessed by the EFSA GMO Panel (2023); with no safety concerns identified. The GMO Panel considers that the applicants should guarantee the timely delivery of potentially relevant information to EFSA to assist in the processing of applications.

The GMO Panel has performed the risk assessment of the new sequencing information for events MIR604 and GA21 in the frame of a request received from the European Commission and concluded that the original risk assessments of events MIR604 and GA21 as a single and as a part of stacked events remains valid (EFSA GMO Panel, 2015d,e) As regards MIR162 (EFSA GMO Panel, 2022a), a difference was located in a cytosine homopolymer region in the second of the two ZmUbiInt promoters contained in the MIR162 insert (bp 6,770–6,782) (EFSA GMO Panel, 2022a). The location of the difference suggests that it is due to the technical difficulties with sequencing the homopolymer regions. The GMO Panel considers that this uncertainty does not raise any safety concern.

Updated bioinformatic analyses for events Bt11, MIR162, MIR604, MON 89034, 5307 and GA21 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 Cry1Ab, Vip3Aa20, mCry3A, Cry1A.105, Cry2Ab2, eCry3.1Ab, mEPSPS, PAT and PMI proteins 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 Bt11, MIR162, MIR604, MON 89034, 5307 and GA21 confirms that the production of a new peptide showing 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 Bt11, MIR162, MIR604, MON 89034, 5307 and GA21 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[7]

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-DE-2018-149. Based on the outcome of the scoping review, the GMO Panel agrees that there is limited value of undertaking a systematic review for maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 at present.

The GMO Panel assessed the applicant's literature searches on the six-event stack maize, which include a scoping review, according to the guidelines given in EFSA (2010, 2019b).

The literature searches provided by the applicant did not identify any relevant publications on maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21. The GMO Panel considered that the search was conducted in line with the applicable guidelines. The GMO Panel noted, however, that a recent European patent of potential relevance to MIR162 was not identified by the literature search (see Section 3.2). The GMO Panel considers that the applicants should complete a comprehensive search of all relevant published literature and patents and guarantee the delivery of potentially relevant scientific information to EFSA to assist in the processing of applications.

3.4. Risk assessment of the six-event stack maize

3.4.1. Molecular characterisation[8]

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 Bt11, MIR162, MIR604, MON 89034, 5307 and GA21 were combined by crossing to produce the stack maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21. The structure of the inserts introduced into the six-event stack maize is described in detail in the respective EFSA scientific opinions (Table 2) and no new genetic modifications were involved. Genetic elements in the expression cassettes of the single events are summarised in Table 3.

Intended effects of the inserts in the six-event stack maize are summarised in Table 4.

Based on the known biological function of the newly expressed proteins (Table 4), the only foreseen interactions at the biological level are between the Cry proteins or between the Vip3Aa20 and the Cry proteins, which will be dealt with in Sections 3.4.4.

Table 3: Genetic elements in the expression cassettes of the events stacked in maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
Bt11	35S (CaMV)*	IVS6 (*Zea mays*)	–	*cry1Ab* (*Bacillus thuringiensis*)	*nos* (*Agrobacterium tumefaciens)*
	35S (CaMV)	IVS2 (*Zea mays*)	–	*pat* (*Streptomyces viridochromogenes*)	*nos* (*Agrobacterium tumefaciens*)

[7] Dossier: Part II – Section 7; additional information: 20/2/2019, 9/9/2022 and 16/2/2023.
[8] Dossier: Part II – Section 1.2; additional information: 28/2/2019, 13/6/2019, 20/9/2019, 22/7/2020, 9/9/2022 and 17/10/2022.

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
MIR162	ZmUbiInt (*Zea mays*)	–	–	*vip3Aa20* (*Bacillus thuringiensis*)	35S (CaMV)
	ZmUbiInt (*Zea mays*)	–	–	*pmi* (*Escherichia coli*)	*nos* (*Agrobacterium tumefaciens)*
MIR604	MTL (*Zea mays*)	–	–	*mcry3A* (*Bacillus thuringiensis*)	*nos* (*Agrobacterium tumefaciens*)
	ZmUbiInt (*Zea mays*)	–	–	*pmi* (*Escherichia coli*)	*nos* (*Agrobacterium tumefaciens*)
MON 89034	35S (CaMV)	*cab* (*Triticum aestivum*)	–	*cry1A.105* *(Bacillus thuringiensis)* *cry2Ab2*	*hsp17* (*Triticum aestivum*)
	35S (FMV)	–	CTP (*Zea mays*)	*(Bacillus thuringiensis)*	*nos* (*Agrobacterium tumefaciens)*
5307	CMP (CmYLCV)	–	–	*ecry3.1Ab* (*Bacillus thuringiensis)*	*nos* (*Agrobacterium tumefaciens*)
	ZmUbiInt (*Zea mays*)	–	–	*pmi* (*Escherichia coli*)	*nos* (*Agrobacterium tumefaciens*)
GA21	actin 1 (*Oryza sativa*)	actin 1 (*Oryza sativa*)	OTP (*Helianthus annuus*)	m*epsps* (*Zea mays*)	*nos* (*Agrobacterium tumefaciens*)

UTR: untranslated region.
–: When no element was specifically introduced to optimise expression.
*: Source of genetic material.

Table 4: Characteristics and intended effects of the events stacked in maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21

Event	Protein	Donor organism and biological function	Intended effects in GM plant
Bt11	Cry1Ab	Based on genes from *Bacillus thuringiensis* var. *kurstaki* HD-1. *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 Bt11 expresses a chimeric, truncated *cry1Ab* gene. Cry1Ab is a chimeric 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 Bt11 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 (Vip) (Fang et al., 2007).	Event MIR162 expresses a modified version of the *B. thuringiensis* vip3Aa1 gene, and encodes Vip3Aa20, a protein toxic to certain lepidopteran larvae feeding on maize.

Event	Protein	Donor organism and biological function	Intended effects in GM plant
	PMI[(a)]	Based on a gene from *E. coli*. The phosphomannose isomerase (PMI) enzyme catalyses the isomerisation of mannose-6-phosphate to fructose-6-phosphate and plays a role in the metabolism of mannose (Markovitz et al., 1967).	Event MIR162 expresses PMI, which is used as a 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).
MIR604	mCry3A	Based on genes from *Bacillus thuringiensis* subsp. *tenebrionis*. *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 MIR604 expresses a modified version of the native Cry3A protein (Chen and Stacey, 2003). mCry3A is a protein toxic to certain coleopteran larvae feeding on maize.
	PMI[(a)]	Based on a gene from *E. coli*. PMI (phosphomannose isomerase) 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 MIR604 expresses PMI, which is used as a selectable marker. Mannose normally inhibits root growth, respiration and germination. Transformed cells expressing PMI are able to utilise mannose as a carbon source (Negrotto et al., 2000).
MON 89034	Cry1A.105	Based on genes from *Bacillus thuringiensis* subsp. *kurstaki* and subsp. *aizawai*. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (cry) genes (Schnepf et al., 1998, Ellis et al., 2002).	Event MON 89034 expresses a modified version of the Cry1A-type protein. Cry1A.105 is a protein toxic to certain lepidopteran larvae feeding on maize.
	Cry2Ab2	Based on a gene from *Bacillus thuringiensis* subsp. *kurstaki*. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (cry) genes (Schnepf et al., 1998, Ellis et al., 2002).	Event MON 89034 expresses the Cry2Ab2, a protein toxic to certain lepidopteran larvae feeding on maize.
5307	eCry3.1Ab	Based on genes from *Bacillus thuringiensis* subsp. *kurstaki* and subsp. *tenebrionis*. *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 5307 expresses the synthetic protein eCry3.1Ab which is a chimera composed of the N-terminal portion of the mCry3A and the C-terminal portion of the Cry1Ab protein. eCry3.1Ab is an insecticidal protein toxic to certain coleopteran larvae feeding on maize.
	PMI	Based on a gene from *E. coli*. PMI (phosphomannose isomerase) 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 5307 expresses PMI, which is used as a 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).
GA21	mEPSPS	Based on a gene from *Zea mays*. 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 GA21 expresses mEPSPS protein which is a modified version of the endogenous EPSPS enzyme that confers tolerance to glyphosate-based herbicides (Lebrun et al., 2003).

(a): The PMI protein is expressed in events MIR604, MIR162 and 5307. Event MIR604 expresses PMI, differing from PMI expressed in events MIR162 and 5307 by two amino acids. The PMI from the three events hereafter referred to as PMI.

3.4.1.2. Integrity of the events in the six-event stack

The genetic stability of the inserted DNA over multiple generations in the single maize events Bt11, MIR162, MIR604, MON 89034, 5307 and GA21 was demonstrated previously (see Table 2). Integrity of these events in the six-event stack maize was assessed by PCR, Sanger and NGS sequence analyses showing that the sequences of the events (inserts and their flanking regions) in the six-event maize stack are identical to the sequences already assessed (see Table 2 and Section 3.2) for the six single events.

As regards MIR162, a potential sequence difference was located in a cytosine homopolymer region in the second of the two ZmUbiInt promoters contained in the MIR162 insert (bp 6,770–6,782) driving the expression of the *pmi* gene. The location of the difference in the homopolymer region suggests that it is due to the technical difficulties with sequencing. Taken together the data above confirm that the integrity of these events was maintained in the six-event stack maize.

3.4.1.3. Information on the expression of the insert

Cry1Ab, Vip3Aa20, mCry3A, Cry1A.105, Cry2Ab2, eCry3.1Ab, mEPSPS, PAT and PMI protein levels were analysed by enzyme-linked immunosorbent assay (ELISA), in material harvested in a field trial across 3 locations in the USA in 2015. Samples analysed included leaves, roots and whole plant (all at BBCH 16 and BBCH 63–65[9]), pollen (BBCH 63–65) and grain (BBCH 87–99 and senescence), not treated with the intended herbicides. In order to assess changes in protein expression levels, which may result from potential interactions between the events, protein levels were determined for the six-event stack maize and the corresponding single events in different parts of the plant.

The levels of all the newly expressed proteins in the six-event stack maize and the corresponding singles were comparable in all tissues, except for PMI protein levels that are expected to be different because of the combination of events 5307, MIR162 and MIR604 all three producing PMI in the six-event stack maize (Appendix B). Therefore, there is no indication of an interaction that may affect the levels of the newly expressed proteins in this stack.

3.4.1.4. Conclusion on molecular characterisation

The molecular data establish that the events stacked in maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins are similar in the six-event stack maize and in the single events, except PMI that shows the expected higher level in the stack resulting from the combination of events 5307, MIR162 and MIR604. 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 functions of the newly expressed proteins (Table 3), the only foreseen interactions at the biological level are between the Cry proteins or between the Vip3Aa20 and the Cry proteins, which will be dealt with in Section 3.4.4.

3.4.2. Comparative analysis[10]

3.4.2.1. Overview of studies conducted for the comparative analysis

Application EFSA-GMO-DE-2018-149 presents data on agronomic and phenotypic characteristics, as well as on forage and grain composition of maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 (Table 5).

Table 5: Overview of the comparative analysis studies to characterise the six-event stack maize provided in application EFSA-GMO-DE-2018-149

Study focus	Study details	Comparator	Non-GM reference varieties
Agronomic, phenotypic and compositional analysis	Field study, USA, 2015, nine sites[(a)]	NP2222 × NP2377	6[(b)]

[9] BBCH scale describes phenological stages (Meier, 2001). BBCH16, BBCH63-65 and BBCH87-99 correspond to approximately V6, R1 and R6 stages of maize development, respectively.

[10] Dossier: Part II – Section 1.3; additional information: 21/9/2018, 13/6/2019 and 22/7/2019.

GM: Genetically modified.
(a): The field trials were located in Kimballton, IA; Larned, KS; Wyoming, IL; Germansville, PA; York, NE; Richland, IA; Delavan, WI; Stewardson, IL and Cooper, IA. The field trial in Larned, KS was compromised by early-season herbicide drift from an adjacent field; however, valid data on early stand count was collected and included in the statistical analysis (so that there were data from a total of 10 dataset for early stand count). This field trial was then replanted and added to the field study. A field trial located in Carlyle, IL was excluded from the statistical analysis due to heavy precipitation, which caused uneven germination across the entire trial.
(b): The non-GM maize reference varieties used in the field trials, with comparative relative maturity in brackets, were: SY Generoso (113–115); SY Provial (105–107); Cisko (99–104); SY Sincero (108–112); NK Octet (93–98); and NK Lucius (93–98).

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[11] replicates: the six-event stack maize not exposed to the intended herbicides, the six-event stack maize exposed to the intended herbicides, the comparator NP2222 × NP2377 and three non-GM commercial 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 the six-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).[12]

3.4.2.3. Suitability of selected test materials

3.4.2.3.1. Selection of the test materials

To obtain the six-event stack maize, the previously obtained single events (Bt11, MIR162, MIR604, MON 89034, 5307 and GA21) were transferred by backcrossing in two different non-GM maize inbred lines, NP2222 and NP2377.[13] In subsequent subsections, the six-event stack GM maize refers to hybrid (F_1) obtained crossing GM inbred line NP2222 (carrying Bt11, MIR162, MIR604, 5307 and GA21) with GM inbred line NP2377 (carrying MON 89034).

The comparator used in the field trials is the non-GM maize hybrid NP2222 × NP2377, which has genetic background similar to that of the six-event stack maize (as documented by the pedigree and by the additional information), and is considered to be an appropriate comparator.

The six-event stack maize and the comparator, both with a comparative relative maturity (CRM) of 105–107, which is considered appropriate for growing in environments across North America, where the comparative field trials were conducted.

The six non-GM reference hybrid varieties with a CRM ranging from 93 to 115 were selected by the applicant and, at each selected site, three reference varieties were tested (see Table 5). On the basis of the provided information on relative maturity classes and year of registration, the GMO Panel considers the selected non-GM reference hybrid varieties appropriate for the comparative assessment.

3.4.2.3.2. Seed production and quality

Seeds of the six-event stack maize and the non-GM comparator NP2222 × NP2377 used in the 2015 field trials were produced from plants free of diseases, 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 seeds were tested for their germination capacity under warm and cold temperature conditions.[14] Germination capacity of the six-event stack maize and its non-GM comparator were compared for germination capacity and the results[15] indicate that the seed germination of the

[11] A fifth replicate was used as backup if samples from other replicates had been lost. Agronomic and phenotypic data were collected and analysed.
[12] 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).
[13] The single events were originally obtained in diverse genetic backgrounds and were backcrossed to stabilise the events into the two selected non-GM inbred lines. These were then crossed to obtain the GM hybrid line used in the comparative analysis.
[14] The seed germination test reports were produced by the North Carolina Department of Agriculture. Warm temperature condition corresponds to 25°C for 6 days and cold temperature to 10°C for 7 days followed by 5 days at 23°C.
[15] GM hybrid maize showed a mean germination of 96% and 46% while the non-GM comparator showed a mean of 97% and 43% under warm and cold temperature conditions, respectively.

six-event stack maize was not different than that of its non-GM comparator. The GMO Panel considers that the starting seed used as test material in the agronomic, phenotypic and compositional studies was of acceptable quality.

3.4.2.3.3. Conclusion on suitability

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

3.4.2.4.2. Meteorological conditions

Maximum and minimum mean temperatures and sum of precipitations were provided on a weekly basis. No exceptional weather conditions were reported at any of the selected sites; therefore, the GMO Panel considers that the meteorological dataset falls within the historical range of climatic conditions normally occurring at these sites.

3.4.2.4.3. Management practices

The field trials included plots containing the six-event stack maize, plots with the comparator and plots with non-GM maize reference varieties, mostly managed according to local agricultural practices. In addition, the field trials included plots containing six-event stack maize managed following the same agricultural practices, plus exposed to two sequential treatments with glyphosate=containing herbicides at BBCH 13–14 growth stage[17] and with a glufosinate-ammonium-containing herbicide at BBCH 15–16 growth stage.

At some field trial sites,[18] sowing occurred later than usual (close to the limit of the typical range), resulting in a shorter and/or shifted growing cycle. The additional information indicated that the shorter and/or shifted growing cycle was unlikely to affect the representativeness of field trial conditions. In addition, despite not considered a normal agricultural practice, thinning was applied at all field trial sites to achieve a more homogeneous plant density across plots.

Despite late sowing and thinning represent deviations from standard management practices under farm cultivation, those agronomic practices do not alter the capability to conclude on the comparative assessment.

3.4.2.4.4. Conclusion on representativeness

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

3.4.2.5. Agronomic and phenotypic analysis

Ten agronomic and phenotypic endpoints[19] plus information on abiotic stressors, disease incidence and insect damage were collected from the field trials (see Table 5).

The GMO Panel noted that the endpoint ear count, which is part of the minimum set of endpoints defined in EFSA GMO Panel (2015a), had not been measured in the field trials. The applicant justified this exclusion as the result of a failed update of study protocols. The applicant also argued that, even

[16] Soil types of the field trials were silt loam, loam, clay loam and silty clay loam; soil organic matter ranged from 1,9% to 5,2%; pH ranged from 5,7 to 6,9; average temperatures and sum of precipitations during the usual crop growing season ranged respectively from 19,5°C to 15,5°C and from 504 mm to 815 mm.

[17] BBCH scale describes phenological stages (Meier, 2001).

[18] Six field trials located in Iowa (three), Kansas, Nebraska and Illinois.

[19] Early stand count, days to 50% pollen shed, days to 50% silking, total lodging, plant height, days to maturity, final stand count, grain moisture, grain yield and 1,000-kernel weight.

though no experimental data were available, a difference in ear count was unlikely considering the results for yield (see below). The GMO Panel requested the applicant to support this conclusion with the assessment of additional, relevant yield components. The applicant provided data on kernels per plant,[20] so that the assessment of the GMO Panel was based on a total of 11 endpoints.

The endpoint lodging could not be statistically analysed as described in Section 3.4.2.2 because of lack of variability in the data. The statistical analysis was applied to the other 10 endpoints, with the following results:

- For the six-event stack maize (not treated with the intended herbicides), the test of difference identified statistically significant differences with the non-GM comparator for early stand count, plant height, grain moisture, 1,000-kernel weight, days to 50% pollen shed, days to 50% silking and days to maturity. All these endpoints fell under equivalence category I.
- For the six-event stack maize (treated with the intended herbicides), the test of difference identified statistically significant differences with the non-GM comparator for early stand count, plant height, grain moisture, 1,000-kernel weight, days to 50% pollen shed and days to 50% silking. All these endpoints fell under equivalence category I.

As there were no data on ear count (see above), an uncertainty in the agronomic and phenotypic characterisation of the six-event stack maize remains. However, considering the lack of differences found for the two relevant yield components (yield and kernels per plant), the GMO Panel considered that the uncertainty related to the lack of ear count data does not alter the suitability of the comparative analysis and its ability to conclude on the agronomic and phenotypic analysis of the six-event stack maize.

3.4.2.6. Compositional analysis

Forage and grain harvested from the field trials (see Table 2) were analysed for 82 different constituents (9 in forage and 73 in grain), including those recommended by the OECD (OECD, 2002). The statistical analysis as described in Section 3.4.2.2 was not applied to 15 grain constituents,[21] because their concentration in more than half of the samples were below the limit of quantification (LOQ), and to moisture levels in grain, as the grains were dried before the analytical measurements.

The statistical analysis was applied to a total of 66 constituents (9 in forage[22] and 57 in grain[23]); a summary of the outcome of the test of difference and the test of equivalence is presented in Table 6:

- For the six-event stack maize not treated with the intended herbicides, statistically significant differences in the comparison with the non-GM comparator were identified for 19 endpoints (all in grain). All these endpoints fell under equivalence category I or II except for levels of neutral detergent fibre (NDF), stearic acid (C18:0), ferulic acid and *p*-coumaric acid which fell under equivalence category III or IV (Table 7).
- For the six-event stack maize treated with the intended herbicides, statistically significant differences with the non-GM comparator were identified for 20 endpoints (all in grain). All these endpoints fell under equivalence category I or II except for levels of NDF, stearic acid (C18:0), ferulic acid and *p*-coumaric acid which fell under equivalence category III or IV (Table 7).

[20] Kernels per plant was not directly measured but calculated from three other endpoints as kernels per plant = 1000 × yield/(1000-kernel weight × final stand count).

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

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

[23] Ash, carbohydrates, fat, protein, starch, acid detergent fibre (ADF), neutral detergent fibre (NDF), total dietary fibre (TDF), calcium, copper, iron, magnesium, manganese, phosphorus, potassium, zinc, alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C21:0), behenic acid (C22:0), α-tocopherol, β-carotene, folic acid, niacin, pyridoxine, riboflavin, thiamine, ferulic acid, *p*-coumaric acid, total inositol, phytic acid, raffinose and trypsin inhibitor.

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

		Test of difference[a]			
		Not treated[c]		Treated[c]	
		Not different	Significantly different	Not different	Significantly different
Test of equivalence[b]	Category I/II	43	15[d]	43	16[d]
	Category III/IV	3[e]	4[f]	2[e]	4[f]
	Not categorised	1[g]	–	1[g]	–
	Total endpoints	66		66	

(a): Comparison between six-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 (glyphosate and glufosinate ammonium).
(d): Endpoints with significant differences between the GM maize and the non-GM comparator and falling in equivalence category I-II. In forage, none. In grain, not treated only: copper and iron. Treated only: fat, tryptophan and palmitic acid (C16:0). Both treated and not treated: ADF, glycine, palmitoleic acid (C16:1), heptadecanoic acid (C17:0), arachidic acid (C20:0), eicosenoic acid (C20:1), behenic acid (C22:0), α-tocopherol, β-carotene, niacin, pyridoxine, thiamine and raffinose.
(e): Endpoints falling in equivalence category III-IV and with no significant differences between the GM maize and the non-GM comparator. In forage, none. In grain, not treated only: serine. Both treated and not treated: aspartic acid and manganese.
(f): Endpoints falling in equivalence category III-IV and with significant differences between the GM maize and the non-GM comparator. In forage, none. In grain (for both treated and not treated GM maize): NDF, stearic acid (C18:0), ferulic acid and *p*-coumaric acid. Quantitative results for these endpoints are reported in Table 7.
(g): Protein levels in forage were not categorised for equivalence; however, no significant differences were identified between the GM maize (treated or not treated) and the non-GM comparator.

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

Table 7: Quantitative results (estimated means and equivalence limits) for compositional endpoints in maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 that are further assessed based on the results of the statistical analysis

	Endpoint	Maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21		Non-GM comparator	Non-GM reference varieties	
		Not treated	Treated[a]		Mean	Equivalence limits
Grain	NDF (% DM)	12.6*	12.5*	11.9	10.0	8.5–11.9
	Stearic acid (C18:0) (% FA)	2.15*	2.18*	2.03	1.88	1.72–2.05
	Ferulic acid (mg/kg)	2,919*	2,925*	2,692	2,041	1,600–2,590
	p-Coumaric acid (mg/kg)	443*	444*	387	181	79–418

DM: dry matter; % FA: percentage total fatty acid.
(a): Not treated: treated only with conventional herbicides. Treated: treated with the intended herbicides (glyphosate and glufosinate ammonium).
*: For the GM maize, significantly different values are marked with an asterisk, while the outcomes of the test of equivalence are differentiated by greyscale backgrounds: light and dark grey backgrounds correspond to equivalence category III and IV, respectively.
Means and equivalence limits were calculated on a log-transformed scale; the values shown in the table are back-transformed to the original scale.

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 are 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 agronomic and phenotypic characteristics tested between maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 and the non-GM comparator needs further assessment for environmental safety.
- None of the differences identified in forage and grain composition between maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 and the non-GM comparator needs further assessment regarding food and feed safety, except for grain levels of NDF, stearic acid (C18:0), ferulic acid and *p*-coumaric acid, which are further assessed in Section 3.4.3.

3.4.3. Food/feed safety assessment[24]

3.4.3.1. Effects of processing

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

3.4.3.2. 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, 2010f, 2011a, 2017a, 2021a). 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 of the most prominent traits attributed to food allergens is protein stability (Helm, 2001; Breiteneder and Mills, 2005; Foo and Mueller, 2021; Costa et al., 2022).

3.4.3.2.1. Effect of temperature and pH on newly expressed proteins

The effects of temperature and pH on the newly expressed Cry1Ab, Vip3Aa20, mCry3A, Cry1A.105, Cry2Ab2, eCry3.1Ab, mEPSPS, PAT 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 Cry1Ab, Vip3Aa20, mCry3A, Cry1A.105, Cry2Ab2, eCry3.1Ab, mEPSPS, PAT 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.

3.4.3.3. Toxicology

3.4.3.3.1. Testing of newly expressed proteins

The Cry1Ab, Vip3Aa20, mCry3A, Cry1A.105, Cry2Ab2, eCry3.1Ab, mEPSPS, PAT and PMI proteins are newly expressed in the six-event stack maize (Section 3.4.1).

The GMO Panel has previously assessed these proteins in the context of the single maize events (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 new information that would change these conclusions. The potential for a functional interaction among the proteins newly expressed in six-event stack maize has been assessed with regard to human and animal health.

The enzymatic proteins (mEPSPS, PAT and PMI) catalyse distinct biochemical reactions, acting on unrelated substrates, and are not expected to interact. The mEPSPS protein confers tolerance to glyphosate-containing herbicides acting on the shikimic acid pathway for the biosynthesis of aromatic

[24] Dossier: Part II – Sections 1.4, 1.5, 1.6, 1.7 and 2; additional information: 2/4/2019, 3/4/2019, 20/6/2019, 30/4/2020, 19/3/2021, 20/8/2021, 29/11/2021, 11/1/2022 and 31/8/2022.

amino acids in plants. The PAT protein confers tolerance to glufosinate ammonium-containing herbicides, acting by acetylation of glufosinate ammonium. The PMI protein is used as a selectable marker and plays a role in the metabolism of mannose in plants, allowing maize cells to use mannose as a sole carbon source.

The insecticidal proteins Cry1Ab, mCry3A, Cry1A.105, Cry2Ab2 and eCry3.1Ab act 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 secreted by *B. thuringiensis* during its vegetative phase acting in target insects via a mechanism similar to that of Cry proteins (Chakroun et al., 2016; Bel et al., 2017). On the basis of the known biological function of the individual newly expressed proteins, there is currently no expectation for possible interactions relevant to the food and feed safety of this six-event stack maize.

The GMO Panel concludes that there are no safety concerns for human and animal health related to the newly expressed proteins Cry1Ab, Vip3Aa20, mCry3A, Cry1A.105, Cry2Ab2, eCry3.1Ab, mEPSPS, PAT and PMI in the six-event stack maize.

3.4.3.4. 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 grain and forage from six-event stack maize. Therefore, no further food/feed safety assessment of components other than the newly expressed proteins is required.

3.4.3.4.1. Information on altered levels of food and feed constituent

Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, no altered levels of food/feed constituents have been identified in grains and forage from the six-event stack maize except for grain levels of NDF, stearic acid (C18:0), ferulic acid and *p*-coumaric acid. 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.

3.4.3.4.2. 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 six-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 the six-event stack maize are not considered necessary by the GMO Panel (EFSA GMO Panel, 2011a).

In accordance with Regulation (EU) No 503/2013, the applicant provided a 90-day feeding study in rats on whole food and feed from each of the maize single events composing this six-event stack maize.

The studies were conducted with three upper limit doses of 50%[25],41.5%[26] and 33%[27]. Since 2019, a 50% maize incorporation rate is used as the high dose (EFSA GMO Panel, 2021c,d, 2022c,d, e). While the GMO Panel is reviewing the evidence regarding test diets incorporating up to 50% and the potential to induce nutritional imbalance, currently the Panel considers that the upper incorporation rates of 41.5% and 33% are acceptable for these existing studies.[28]

[25] 90-day feeding study with maize MIR604 was completed in 2021.

[26] 90-day feeding study with maize Bt11 was completed in 2017; with maize MIR162 in 2006; with maize GA21 in 2005; with maize 5307 in 2011.

[27] 90-day feeding study with maize MON 89034 was completed in 2007.

[28] Recent work (e.g. Steinberg, 2019; 2020) indicates that an acceptable upper limit for incorporation of maize into rodent diets is 50%. Many rodent studies evaluated by the GMO Panel were performed prior to 2019 and used upper incorporation rates of 33% or 41.5%. The GMO Panel considers that a 1.5-fold or 1.2-fold increase in incorporation rate is unlikely to identify any new hazards in the context of this application and therefore there is no reason to repeat these older studies using the new upper incorporation rate of 50%. This approach is consistent with Directive 2010/63/EU on the protection of animals used for scientific purposes.

90-Day feeding studies on maize Bt11, MIR162, MIR604 and MON 89034

The GMO Panel had previously concluded that the 90-day feeding studies with maize Bt11 (EFSA GMO Panel, 2021a,b,c,d), MIR604 (EFSA GMO Panel, 2022c), MIR162 and MON 89034 (EFSA GMO Panel, 2019d) are in line with Regulation (EU) No 503/2013 and do not show adverse effects related to diets incorporating the respective single-events.

90-Day feeding study on maize GA21

A 90-day study on maize GA21 performed in 2005 had been previously assessed by the GMO Panel in the context of the single-event applications (EFSA, 2007; EFSA GMO Panel, 2011c) and no adverse effects related to the administration of the GM diet had been identified. In the context of the assessment of this six-event stack maize, EFSA asked for additional information to confirm the adherence of this study to requirements of Regulation (EU) 503/2013, OECD TG 408 (*OECD,* 1998), EFSA Scientific Committee (2011) and EFSA (2014). The applicant provided details on the appropriateness of the test and control materials and on the experimental design, together with additional statistical analyses. The GMO Panel concludes that this study is in line with the legal requirements and confirms the original conclusions that there are no indications of adverse effects related to the 90-day administration to rats of diets including grains from maize GA21, up to 41.5% of inclusion rate.

90-Day feeding study on maize 5307

In this study, pair-housed Han Wistar rats (10 per sex per group; 2 rats per cage) were randomly allocated to four different groups.

Groups were fed diets containing maize 5307 grains or the non-GM comparator grains, at 10% and 41.5% of inclusion levels.

The study was adapted from OECD test guideline 408 (*OECD,* 1998), aligned with EFSA Scientific Committee guidance (EFSA Scientific Committee, 2011) and complied with the principles of good laboratory practice (GLP) with some minor deviations not impacting the study results and interpretation.

The stability of the test and control materials was not verified; however, in accordance to product expiration declared by the diet manufacturer, the constituents of the diets are considered stable for the duration of the treatment. The GMO Panel considered this justification acceptable. Diet preparation procedures and regular evaluations of the mixing methods guaranteed the homogeneity and the proper concentration of the test or control substances in them.

Event-specific PCR analysis confirmed the presence of the event maize 5307 in the GM grains and excluded the presence of the event in the respective controls. ELISA analyses also confirmed the presence of the event maize 5307 in the GM maize grains and GM diets, and the absence of the of the event in the respective controls.

Both the GM grains and diets were analysed for nutrients, antinutrients and potential contaminants. Balanced diets were formulated based on the specifications for Special Diets Services Limited (SDS) diets.

Feed and water were provided ad libitum. In-life procedures and observations and terminal procedures were conducted in accordance to OECD TG 408 (1998).

An appropriate range of statistical tests were performed on the results of the study. Detailed description of the methodology and of statistically significant findings identified in rats given diets containing grains/meal derived from maize 5307 is reported in Appendix C.

There were no mortalities and no test diet-related clinical signs. No test diet-related adverse findings were identified in any of the investigated parameters. A small number of statistically significant findings were noted but these were not considered adverse effects of treatment for one or more of the following reasons:

- were within the normal variation[29] for the parameter in rats of this age;
- were of small magnitude;
- were identified at only a small number of time intervals with no impact on the overall value;
- exhibited no consistent pattern with related parameters or end-points.
- exhibited no consistency with increasing incorporation levels.

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

The GMO Panel concludes that this study is in line with the requirements of Regulation (EU) No 503/2013 and that no treatment related adverse effects were observed in rats after feeding diets containing maize 5307 grains at 10% or 41.5% for 90 days.

3.4.3.5. Allergenicity

A weight-of-evidence approach was followed for the allergenicity assessment, 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 performed (EFSA GMO Panel, 2017a).

3.4.3.5.1. Assessment of allergenicity of the newly expressed proteins

The GMO Panel has previously evaluated the safety of the newly expressed Cry1Ab, Vip3Aa20, mCry3A, Cry1A.105, Cry2Ab2, eCry3.1Ab, mEPSPS, PAT 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 six-event stack maize that might change the previous conclusions of the GMO Panel has become available. Based on the current knowledge, and as there is no evidence of allergenicity of the newly expressed proteins, there are no expected concerns of allergenicity as a consequence of their presence in this six-event stack maize.

The GMO Panel has previously evaluated the safety of the newly expressed proteins, and no evidence of adjuvanticity were identified in the context of the applications assessed (Table 2). This aspect has been discussed in detail by EFSA (EFSA, 2018; Parenti et al., 2019). To date, there is no evidence for adjuvanticity in the GMOs assessed by the Panel. This six-event stack maize has similar levels of the individual Bt proteins as those in the respective single maize events (see Section 3.4.1). The GMO Panel did not find indications that the Bt proteins at the levels expressed in this six-event stack maize might be adjuvants able to enhance an allergic reaction.

The applicant also provided information on the safety of the Cry1Ab, Vip3Aa20, mCry3A, Cry1A.105, Cry2Ab2, eCry3.1Ab, mEPSPS, PAT 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, 2017). The assessment of the Cry1.Ab, Vip3Aa20, mCry3A, Cry2Ab2, eCry3.1Ab and mEPSPS proteins identified no perfect or relevant partial matches with known celiac disease peptide sequences. The assessment of the PAT, PMI and Cry1A.105 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 (EFSA GMO Panel, 2019a,e, 2021b,d, 2022a,b). Based on additional considerations on the 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, 2017), 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.

[29] Although animals used in a toxicology study are of the same strain, from the same supplier and are closely matched for age and body weight at the start of the study, they exhibit a degree of variability in the parameters investigated during the study. This variability is evident even within control groups. To help reach a conclusion on whether a statistically significant finding in a test group is 'adverse' account is taken of whether the result in the test group is outside the normal range for untreated animals of the same strain and age. To do this, a number of sources of information are considered, including the standardised effect size, the standard deviations and range of values within test and control groups in the study and, if applicable, data from other studies performed in the same test facility within a small timeframe and under almost identical conditions (Historic Control Data).

3.4.3.5.2. 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[30] (OECD, 2002). Therefore, the GMO Panel does not request experimental data to analyse the allergen repertoire of GM maize. In the context of this application and considering the data from the molecular characterisation, the compositional analysis and the assessment of the newly expressed proteins (see Sections 3.4.1, 3.4.2 and 3.4.3), the GMO Panel identifies no indications of a potentially increased allergenicity of food and feed derived from this six-event stack maize with respect to that derived from the comparator and the non-GM reference varieties tested.

3.4.3.6. Dietary exposure assessment to new constituents

In line with Regulation (EU) No 503/2013 the applicant provided dietary exposure estimates to Cry1Ab, Vip3Aa20, mCry3A, Cry1A.105, Cry2Ab2, eCry3.1Ab, mEPSPS, PAT and PMI proteins newly expressed in the six-event stack maize. Dietary exposure was estimated based on protein expression levels reported in this application for the six-event stack maize treated with the intended herbicides, the current available consumption data and feed practices, the foods and feeds currently available in the market and the described processing conditions.

For the purpose of estimating dietary exposure, the levels of newly expressed proteins in the six-event stack maize grains, forage and pollen were derived from replicated field trials (four replicates from three locations, n = 12) in the United States in 2021. Table 8 describes the protein expression levels used to estimate both human and animal dietary exposure.

Table 8: Mean values (n = 12, μg/g dry weight and μg/g fresh weight) for newly expressed proteins in grains, forage and pollen from maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 treated with the intended herbicides[(a)]

Protein	Tissue/developmental stage		
	Grains/BBCH 87 (μg/g fresh weight)	Pollen/BBCH 63–65 (μg/g dry weight)[(b)]	Forage/BBCH 85 (μg/g fresh weight)
Cry1Ab	3.71	< 0.160[(e)]	6.93
PAT[(c)]	< 0.0189[(d)]	< 0.240[(e)]	0.0797
Vip3Aa20	41.8	37.0	25.8
PMI[(f)]	6.19	113	5.85
mCry3A	0.193	0.134	1.10
Cry1A.105	3.82	1.77	2.66
Cry2Ab2	1.51	< 0.438[(e)]	7.54
eCry3.1Ab	1.80	< 2.0[(e)]	2.60
mEPSPS	7.92	147	6.09

(a): Intended herbicides: glufosinate-ammonium and glyphosate herbicides.
(b): Concentrations values in pollen were adjusted to 6% moisture content before using them to estimate dietary exposure to the different newly expressed proteins via the consumption of pollen supplements.
(c): In accordance with EFSA guidance (EFSA, 2019a), the greatest means of newly expressed protein (NEP) concentrations among growth stages of kernels were used to estimate exposures. For PAT protein the selected growth stage was BBCH 99.
(d): When estimating the mean, individual results reported as less than the LOD were replaced by the value reported as the LOD, and results less than the LOQ were replaced by the value reported as the LOQ. For PAT protein all samples were reported as below LOD (0.0189 μg/g fresh weight).
(e): All pollen samples analysed for Cry1Ab protein were below LOQ (0.160 μg/g dry weight); all pollen samples analysed for PAT protein were below LOD (0.240 μg/g dry weight); all pollen samples analysed for Cry2Ab2 protein were below LOQ (0.438 μg/g dry weight); all pollen samples analysed for eCry3.1Ab protein were below LOQ (2 μg/g dry weight).
(f): PMI levels in maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 are a sum of two protein variants; one expressed in MIR162 and 5307 and another expressed in MIR604. These two PMI variants differ by two amino acids, previously assessed by the GMO Panel (Table 2).

[30] 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.

3.4.3.6.1. Human dietary exposure

Chronic and acute estimations of dietary exposure to Cry1Ab, Vip3Aa20, mCry3A, Cry1A.105, Cry2Ab2, eCry3.1Ab, mEPSPS, PAT and PMI proteins newly expressed in the six-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 six-event stack maize grains, a conservative scenario with 100% replacement of conventional maize by the GM maize was considered. Consumption figures for all relevant commodities (e.g. corn flakes, sweet corn, popcorn, etc.) were retrieved from the EFSA Comprehensive European Food Consumption Database (EFSA consumption database).[31] Corn oil, corn starch and corn syrup were excluded from the assessment since no proteins are expected to be present in these commodities.

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.[32] No losses in the newly expressed proteins during processing were considered except for the commodities mentioned above.

The highest acute dietary exposure (high consumers) was estimated in the age class 'Other children' with exposure estimates that ranged between 0.29 μg/kg bw per day for PAT protein and 635.4 μg/kg bw per day for Vip3Aa20 protein. The main 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 0.15 μg/kg bw per day for PAT protein and 341.3 μg/kg bw per day for Vip3Aa20 protein. The main contributor to the exposure in the dietary survey with the highest estimates was corn flakes.

An ad hoc dietary exposure scenario was provided for consumers of pollen supplements under the assumption that these supplements might be made of pollen from the six-event stack maize. Consumption data on pollen supplements are available for few consumers across seven different European countries.[31] The low number of consumers available adds uncertainty to the exposure estimations which should be interpreted with care, 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 0.09 μg/kg bw per day for mCry3A protein to 102.4 μg/kg bw per day for mEPSPS protein, in the elderly population. Similarly, the highest chronic dietary exposure in average consumers would range from 0.06 μg/kg bw per day for mCry3A protein to 68.3 μg/kg bw per day for mEPSPS protein, also in the elderly population.

3.4.3.6.2. Animal dietary exposure

Dietary exposure to Cry1Ab, Vip3Aa20, mCry3A, Cry1A.105, Cry2Ab2, eCry3.1Ab, mEPSPS, PAT and PMI proteins in the six-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 feed, gluten meal, milled by-products, hominy meal, forage/silage and stover). A conservative scenario with 100% replacement of conventional maize products by the six-event stack maize products was considered.

Mean levels (fresh weight) of the newly expressed proteins in grain and forage from the six-event stack maize treated with the intended herbicides used for animal dietary exposure are listed in Table 8.

Mean levels (fresh weight) of the newly expressed proteins in maize gluten feed and gluten meal, hominy meal and milled by-products were calculated to be, respectively, 2.13, 6.38, 1.18 and 0.894-fold than those in grain, and in maize stover 0.861-fold than in forage, based on adjusting

[31] https://www.efsa.europa.eu/en/applications/gmo/tools. From version updated in March 2022.

[32] Example: 100 g of maize bread are made with approximately 74 g of maize flour, and a reverse yield factor of 1.22 from the conversion of maize grains into flour is used. This results in ~37.7 μg of Vip3Aa20 per gram of maize bread as compared to the 41.8 μg/g reported as mean concentration in the maize grains.

factors that take into account the protein content in these feed materials relative to maize grain and forage (see Appendix D – Table D.1), and assuming that no protein is lost during their production/ processing.

The applicant estimated dietary exposure to Cry1Ab, Vip3Aa20, mCry3A, Cry1A.105, Cry2Ab2, eCry3.1Ab, mEPSPS, PAT and PMI proteins via the consumption of maize grains, gluten feed, gluten meal, milled by-products, hominy meal, forage/silage and stover, based on default values for animal body weight, daily feed intake and inclusion rates (percentage) of maize feedstuffs in diets and rations, as provided for the EU by OECD (2013). The total theoretical maximum contribution to the highest exposure to Cry1Ab, Vip3Aa20, mCry3A, Cry1A.105, Cry2Ab2, eCry3.1Ab, mEPSPS, PAT and PMI proteins was taken into account for each feedstuff, according to the *reasonable worst-case diet/feed* (RWCF) approach described by OECD (2013).[33]

Estimated dietary exposure in the concerned animals is reported in Appendix D (Table D.2).

3.4.3.7. Nutritional assessment of endogenous constituents

The intended traits of the six-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 NDF, stearic acid (C18:0), ferulic acid and *p*-coumaric acid (all in both treated and not treated plants with the intended herbicides) were significantly different from the comparator and showed a lack of equivalence with the set of non-GM reference varieties (Section 3.4.2.6). The biological relevance of these compounds, the role of the six-event stack maize as contributor to their total intake, and the magnitude and direction of the observed changes were considered during the nutritional assessment.

3.4.3.7.1. Human nutrition

In the context of human nutrition, fibre is referred to as dietary fibre, which primarily includes non-starch polysaccharides (mainly cellulose, hemicelluloses, pectins and other hydrocolloids) and lignin EFSA NDA Panel (2010). Consequently, the minor observed increase (~ 6%) in NDF (lignin, hemicellulose and cellulose) implies an increased intake of dietary fibre. No tolerable upper intake level (UL) is derived for dietary fibre and, on contrary, there are nutritional recommendations to increase its intake levels based on its key role on bowel function (EFSA NDA Panel, 2010). Based on this, the GMO Panel considers that the observed increase in NDF in the six-stack maize does not represent any nutritional concern in humans.

Stearic acid is one the most commonly consumed saturated fatty acids together with palmitic acid (C16:0). Stearic acid is a minor fatty acid in maize oil representing ~ 2% of the total fatty acids. The levels of stearic acid in grains from the six-stack maize (treated) were ~ 7% higher as compared to those in the conventional counterpart. After considering the extent of this increase and the limited role of maize and maize-based products as a source of stearic acid in the human diet, the GMO Panel concludes that the increase of stearic acid does not raise any nutritional concern.

Ferulic acid and *p*-coumaric acid are the main phenolic acids in maize grain (OECD, 2002; Zavala-López et al., 2020). It is important to note that ferulic acid and *p*-coumaric acid are common in plant cell wall polysaccharides, therefore significant intake from other dietary sources is expected. Animal studies show very low toxicity for ferulic acid (Mancuso and Santangelo 2014) and no toxicity has been reported in humans for any of them. Therefore, the GMO Panel concludes that the increase of ~9% and ~ 15% of ferulic acid and *p*-coumaric acid respectively, is not relevant from a nutritional point of view for humans.

3.4.3.7.2. Animal nutrition

In the context of animal nutrition, NDF can be regarded as a measure of the plant cell wall material, consisting mainly of lignin, cellulose and hemicellulose. A ruminant's diet consists, in particular, of plants and their by-products containing variable amounts of these fibres, used as energy source by rumen microbes. The limiting factor of fibre digestibility in ruminants is the excessive presence of lignin, which makes cellulose and hemicellulose less available by combining with them. However, the minimal differences of the total NDF observed between the six-stack maize and the conventional counterpart (~ 6%) and the reference varieties do not have biological significance. In contrast, monogastric animals cannot use the fibres as an energy source, because they lack gastric bacterial fermentation and do not have the endogenous enzymes capable of digesting fibre. However,

[33] A full description of the model applied was provided in the study report #RIR-0001899.

some of these fibres can be digested by microbes present in the large intestine. Even in monogastric animals, the minimal differences observed with the conventional counterpart (~ 6%) and the reference varieties do not have a biological significance.[34] The GMO Panel considers that the observed increase in NDF in the six-event stack maize grains does not represent a nutritional concern in animals.

Stearic acid is not an essential fatty acid for animals. The main dietary source of stearic acid is animal fat, while the levels are usually low in vegetable oil, with some exception (e.g. coconut oil, cocoa butter). Stearic acid in maize oil represents a minor fraction (~ 2%) of the total fatty acids, underlying the limited role of maize and maize-based products as a source of stearic acid in the animal diet. The GMO Panel considers that the observed increased in stearic acid in the six-event stack maize grains does not represent any nutritional concern in animals.

Ferulic and *p*-coumaric acids are not considered major elements in animal nutrition. They are the main phenolic acids in maize grain (OECD, 2002; Zavala-López et al., 2020), but other sources of ferulic acid and *p*-coumaric acid in animal nutrition are normally used, such as grain brans and sugar beet pulp. Furthermore, ferulic and *p*-coumaric acids are structural and functional components of plant cells and, therefore, intake from other dietary sources is also expected. The GMO Panel considers that the observed increased in ferulic and *p*-coumaric acids in the six-event stack maize grains does not represent any nutritional concern in animals.

3.4.3.8. Conclusions on the food/feed safety assessment

The newly expressed proteins Cry1Ab, Vip3Aa20, mCry3A, Cry1A.105, Cry2Ab2, eCry3.1Ab, mEPSPS, PAT and PMI in the six-event stack maize do not raise safety concerns for human and animal health. No interactions between the newly expressed proteins relevant for food and feed safety were identified, and no overall toxicological concerns on the six-event stack maize were identified. Moreover, the GMO Panel did not identify indications of safety concerns regarding allergenicity or adjuvanticity related to the presence of the newly expressed proteins in maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21, or regarding the overall allergenicity of this six-event stack maize. Based on the outcome of the comparative assessment and the nutritional assessment, the GMO Panel concludes that the consumption of maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 does not represent any nutritional concern, in the context of the scope of this application.

3.4.4. Environmental risk assessment[35]

Considering the scope of application EFSA-GMO-DE-2018-149, which excludes cultivation, the environmental risk assessment (ERA) of maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 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); (2) the accidental release into the environment of GM material including viable six-event stack maize grains during transportation and/or processing (EFSA GMO Panel, 2010a).

3.4.4.1. Persistence and invasiveness of the GM plant

Maize is highly domesticated, not winter hardy in colder regions of Europe, and generally unable to survive in the environment without appropriate management. 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 (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 six-event stack maize will provide a selective advantage to maize plants, except when they are exposed to glyphosate- and/or glufosinate-ammonium-containing herbicides or infested by insect pests that are susceptible to the *Bt* proteins expressed by the six-event stack maize. However, this fitness advantage will not allow the six-event stack maize to overcome

[34] E.g. 'Feed Tables' – https://www.feedtables.com/content/maize report variability in maize grain NDF mean values, the average value is reported as 12.5% dry matter (DM), and the range is from 7.7% to 16.5% DM, data based on 149 counts.
[35] Dossier: Part II – Section 5.

other biological and abiotic factors (described above) limiting plant's persistence and invasiveness. Therefore, the presence of the intended traits and the observed differences in root lodged plants will not affect the persistence and invasiveness of the GM plant.

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

3.4.4.2. Potential for gene transfer

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

3.4.4.2.1. 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 bioinformatic analyses for events Bt11, MIR162, MIR604, MON 89034, 5307 and GA21 confirm the assessments provided in the context of previous applications (EFSA GMO Panel, 2019c, 2022b,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 six-event stack maize to bacteria does not raise any environmental safety concern.

3.4.4.2.2. Plant-to-plant gene transfer

The potential for occasional feral maize six-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-DE-2018-149 into account (no cultivation), potential interactions of occasional feral six-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 material or occasional feral GM maize plants arising from spilled six-event stack maize grains is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM maize, the GMO Panel considers that potential interactions of the six-event stack maize with non-target organisms do not raise any environmental safety concern. Interactions that may occur between the insecticidal proteins will not alter this conclusion.

3.4.4.5. Interactions with abiotic environment and biogeochemical cycles

Given that environmental exposure to spilled material or occasional feral six-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, the GMO Panel considers that potential interactions with the abiotic environment and biogeochemical cycles do not raise any environmental safety concern.

3.4.4.6. Conclusion of the environmental risk assessment

The GMO Panel concludes that it is unlikely that maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 would differ from conventional maize varieties in its ability to persist under European environmental conditions. Considering the scope of application EFSA-GMO-DE-2018-149, interactions of occasional feral six-event stack maize plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from six-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 six-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

Under the scope of this application (see Table 2), one subcombination has been previously assessed and is discussed in Section 3.5.1 while the subcombinations that have not been previously assessed are discussed in Section 3.5.2.

Literature searches covering the 10 years before submission of the application and the period since the time of validity of the application (January 2007–May 2022) revealed no new scientific information relevant to the risk assessment of this maize stack. Novel information, identified in an independent public consultation and potentially relevant for all subcombinations which contain MIR162, was assessed in EFSA GMO Panel (2023) and found not to raise safety concerns (see Section 3.2).

3.5.1. Subcombination previously assessed

Among the subcombinations covered by the scope of this application, one[36] has been previously assessed by the GMO Panel and no safety concerns were identified (see Table 2). The GMO Panel considers that its previous conclusion on this subcombination remains valid.

3.5.2. Subcombinations not previously assessed

29 of the 30 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,[37] the risk assessment takes as its starting point the assessment of the single maize events, and uses the data generated for the six-event stack as well as all the additional data available on subcombinations previously assessed by the GMO Panel (Table 2) and the additional studies provided by the applicant (Appendix A).

[36] The subcombinations independently of their origin and previously assessed by the GMO Panel that can be obtained from the six-event stack maize are 27, however, the scope of the application covers only one of those (i.e. MON 89034 × MIR162) (EFSA GMO Panel 2019d,e;f; 2022b; 2023).

[37] 115th GMO Panel meeting (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-DE-2018-149

Degree of stacking	Events
Five-event stack	MIR604 × GA21 × MON 89034 × MIR162 × 5307
	Bt11 × GA21 × MON 89034 × MIR162 × 5307
	Bt11 × MIR604 × MON 89034 × MIR162 × 5307
	Bt11 × MIR604 × GA21 × MON 89034 × 5307
	Bt11 × MIR604 × GA21 × MON 89034 × MIR162
Four-event stack	GA21 × MON 89034 × MIR162 × 5307
	MIR604 × MON 89034 × MIR162 × 5307
	MIR604 × GA21 × MON 89034 × 5307
	MIR604 × GA21 × MON 89034 × MIR162
	Bt11 × MON 89034 × MIR162 × 5307
	Bt11 × GA21 × MON 89034 × 5307
	Bt11 × GA21 × MON 89034 × MIR162
	Bt11 × MIR604 × MON 89034 × 5307
	Bt11 × MIR604 × MON 89034 × MIR162
	Bt11 × MIR604 × GA21 × MON 89034
Three-event stack	MON 89034 × MIR162 × 5307
	GA21 × MON 89034 × 5307
	GA21 × MON 89034 × MIR162
	MIR604 × MON 89034 × 5307
	MIR604 × MON 89034 × MIR162
	MIR604 × GA21 × MON 89034
	Bt11 × MON 89034 × 5307
	Bt11 × MON 89034 × MIR162
	Bt11 × GA21 × MON 89034
	Bt11 × MIR604 × MON 89034
Two-event stack	MON 89034 × 5307
	GA21 × MON 89034
	MIR604 × MON 89034
	Bt11 × MON 89034

3.5.2.1. Stability of the events

The genetic stability of the inserted DNA over multiple generations in the six single maize events was demonstrated previously (see Table 2 and Section 3.2). Integrity of the events was demonstrated in maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 (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 six events by 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 29 subcombinations compared with those in the single maize events. This assumption was confirmed by comparing the levels of the newly expressed proteins of each single maize event with those of the six-event stack maize. The levels were similar in the six-event stack maize and in the single events except for the PMI proteins, which showed, in general, the expected higher level in the stack resulting from the combination of the single events MIR162, MIR604 and 5307 (Section 3.4.1.3 and Appendix B). This supports the conclusion that interactions affecting the expression levels of the newly expressed proteins are not expected in the 29 subcombinations not previously assessed and included in the scope of application EFSA-GMO-DE-2018-149.

3.5.2.3. Potential functional interactions among the events

The GMO Panel assessed the potential for interactions among maize events in the 29 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. The GMO Panel took into account all the intended and potential unintended effects considered in the assessment of the six single events, the previously assessed subcombinations (Table 2) and the six-event stack maize. It is concluded that none of these events would raise safety concerns when combined in any of these maize subcombinations. The GMO Panel considers that no further data are needed to complete the assessment of subcombinations from the six-event stack maize.

3.5.3. Conclusion

Since no new safety concerns were identified for the previously assessed subcombinations, the GMO Panel considers that its previous conclusions on these maize subcombinations remain valid. For the remaining 29 subcombinations included in the scope of application EFSA-GMO-DE-2018-149, 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 six-event stack maize.

3.6. Post-market monitoring

3.6.1. Post-market monitoring of GM food/feed

The GMO Panel concluded that maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21, 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). Twenty-seven 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 (29) are expected to be as safe as the single maize events, the previously assessed maize subcombinations and the six-event stack maize (Section 3.5.2). Therefore, the GMO Panel considers that post-market monitoring (PMM) of food and feed from the six-event stack maize and 30 subcombinations, as described in this application, is not necessary.

3.6.2. Post-market environmental monitoring[38]

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 maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for six-event stack maize includes: (1) the description of a monitoring approach involving operators (federations involved in import and processing), reporting to the applicant, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment; (2) a coordinating system established by 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 for the duration of the authorisation period.

[38] Dossier: Part II – Section 6; spontaneous information 31/8/2022.

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

The PMEM plan and reporting intervals are in line with the intended uses of the six-stack maize and 30 subcombinations.

3.6.3. Conclusions on post-market monitoring

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

4. Overall conclusions

The GMO Panel was asked to carry out a scientific assessment of maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 and 30 subcombinations for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003.

No new information was identified on the six single maize events (Bt11, MIR162, MIR604, MON 89034, 5307, GA21) that would lead to a modification of the original conclusions on their safety. The GMO Panel noted, however, that the applicant did not inform EFSA of the existence of a patent of potential relevance to the safety of maize MIR162. The patent was independently identified in a public consultation and assessed by the GMO Panel; no safety concerns were identified. The GMO Panel considers that the applicants should guarantee the timely delivery of potentially relevant scientific information to EFSA to assist in the processing of applications.

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 six-event stack maize does not give rise to food/feed safety and nutritional concerns. The GMO Panel concludes that the six-event stack maize, as described in this application, does not raise any nutritional concern and is as safe as its comparator and the selected non-GM reference varieties.

The GMO Panel concludes that there is a very low likelihood of environmental effects resulting from the accidental release of viable grains from the six-event stack maize into the environment. Since no new data were identified on the 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 29 subcombinations included in the scope of application EFSA-GMO-DE-2018-149, protein expression data for two subcombinations (i.e. Bt11 × MIR162 × MON 89034 and Bt11 × MIR162 × MON 89034 × GA21) has been provided (see Appendix A). The GMO Panel assessed the possible interactions between the events in these subcombinations and concludes that these combinations of events Bt11, MIR162, MIR604, MON 89034, 5307, GA21 would not raise safety concerns. These subcombinations are therefore expected to be as safe as the maize single events, the previously assessed subcombinations and the six-event stack maize.

In addition, the GMO Panel considered the additional unpublished studies listed in Appendix A and a patent, owned by the applicant, identified in an independent public consultation (see above). This new information does not raise any concern for human and animal health and the environment regarding the six-event stack maize and 30 subcombinations. Given the absence of safety and nutritional concerns for foods and feeds from the six-event stack maize and 30 subcombinations, the GMO Panel considers that PMM of these products is not necessary. The PMEM plan and reporting intervals are in line with the intended uses of the six-event stack maize and 30 subcombinations. In conclusion, the GMO Panel considers that maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 and 30 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 Germany received on 13 April 2018 concerning a request for authorisation of the placing on the market of genetically modified maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 submitted in accordance with Regulation (EC) No 1829/2003 by Syngenta Crop Protection NV/SA (EFSA Ref. EFSA-GMO-DE-2018-149; EFSA-Q-2018-00292).

- The application was made valid on 6 July 2018.
- Additional information (1) was requested on 20 July 2018.
- Additional information (1) was received on 21 September 2018.
- Additional information (2) was requested on 27 July 2018.
- Additional information (2) was received on 1 February 2019.
- Additional information (3) was requested on 12 October 2018.
- Additional information (3) was received on 28 February 2019.
- Additional information (4) was requested on 20 November 2018.
- Additional information (4) was received on 25 January 2019.
- Additional information (5) was requested on 18 January 2019.
- Additional information (5) was received on 4 April 2019.
- Additional information (6) was requested on 13 February 2019.
- Additional information (6) was received on 3 April 2019.
- Additional information (7) was requested on 11 April 2019.
- Additional information (7) was received on 13 June 2019.
- Additional information (8) was requested on 21 June 2019.
- Additional information (8) was received on 22 July 2019.
- Additional information (9) was requested on 29 July 2019.
- Additional information (9) was received on 20 September 2019 partial; 30 April 2020 complete.
- Additional information (10) was requested on 29 May 2020.
- Additional information (10) was received on 22 July 2020.
- Additional information (11) was requested on 25 August 2020.
- Additional information (11) was received on 5 August 2020 partial; 20 August 2021 complete.
- Additional information (12) was requested on 9 February 2021.
- Additional information (12) was received on 19 March 2021 partial; 31 August 2022 complete.
- Additional information (13) was requested on 7 October 2021.
- Additional information (13) was received on 29 November 2021.
- Additional information (14) was requested on 4 January 2022.
- Additional information (14) was received on 11 January 2022.
- Additional information (15) was requested on 2 September 2022.
- Additional information (15) was received on 9 September 2022.
- Additional information (16) was requested on 5 October 2022.
- Additional information (16) was received on 6 October 2022.
- Additional information (17) was received on 16 February 2023.
- Supplementary information was provided on voluntary basis on 20 June 2019; 31 August 2022 and 17 October 2022.

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Abbreviations

ADF	acid detergent fibre
bw	body weight
bp	base pair
CaMV	cauliflower mosaic virus
CRM	comparative relative maturity
CTP	chloroplast transit peptide
DM	dry matter
dw	dry weight
ELISA	enzyme-linked immunosorbent assay
FA	fatty acid
GLP	good laboratory practice
GM	genetically modified
GMO	genetically modified organism
GMO Panel	EFSA Panel on Genetically Modified Organisms
HGT	horizontal gene transfer
HR	homologous recombination
hsp	heat shock proteins
LOD	limit of detection
LOQ	limit of quantification
mEPSPS	modified 5-enolpyruvylshikimate-3-phosphat synthase
MS	Member States
NEP	newly expressed protein
NDF	neutral detergent fibre
nos	nopaline synthase
OECD	Organisation for Economic Co-operation and Development
OTP	optimised transit peptide
ORFs	open reading frames
PAT	phosphinothricin-acetyl-transferase
PMEM	post-market environmental monitoring
PMI	phosphinothricin acetyl transferase
PMM	post-market monitoring

SDS	Special Diets Services
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 Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 for humans, animal or the environment.

Study identification	Title
TK0220412 A1	Bt11 × MIR162 × MON 89034 × GA21 Maize: Comparative Southern Blot Analyses
TK0244501	Bt11 × MIR162 × MON 89034 Maize: Comparative Southern Blot Analyses
TK0250046	Quantitative Analysis of Transgenic Proteins in Bt11 × MIR162 × MON 89034 × 5307 × GA21 Maize, Bt11 × MIR162 × MON 89034 Maize, and Component Maize Events Bt11, MIR162, MIR604, MON 89034, 5307, and GA21
TK0259988_SR_02	Comparison of Transgenic Protein Concentrations in Bt11 × MIR162 × MON 89034 Maize and Component Maize Events Bt11, MIR162, and MON 89034
TK0220411 A2	Comparison of Transgenic Protein Concentrations in Bt11 × MIR162 × MON 89034 × GA21, Event Bt11, Event MIR162, Event MON 89034, and Event GA21 Maize Tissues
TK0250045	Evaluation of Agronomic Characteristics and Collection of Forage and Grain Samples in Preparation for Compositional Analysis of Bt11 × MIR162 × MON 89034 × 5307 × GA21 Maize and Bt11 × MIR162 × MON 89034 Maize Grown in the USA in 2015
TK0220353	Compositional Analysis of Forage and Grain from Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 Maize Grown During 2015 in the USA
TK0220349	Collection of Agronomic Data and Forage and Grain Samples for Compositional Analysis of Maize Event 3272, Bt11 × MIR162 × MON 89034 × GA21 Maize, Bt11 × MIR162 Maize, and Bt11 × TC1507 × GA21 Maize Grown in the USA in 2014
TK0220405	Agronomic Performance of Bt11 × MIR162 × MON 89034 × GA21 Maize Grown in the USA in 2014
TK0220408	Compositional Analysis of Forage and Grain from Bt11 × MIR162 × MON 89034 × GA21 Maize Grown During 2014 in the USA
TK0244496	Agronomic Performance of Bt11 × MIR162 × MON 89034 Maize Grown in the USA in 2015
TK0244498	Compositional Analysis of Forage and Grain from Bt11 × MIR162 × MON89034 Maize Grown During 2015 in the USA
TK0220350 A1	Agronomic Performance of Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 Maize Grown in the USA in 2015
TK0220394	Investigation of the Potential Interaction between Lepidopteran-active Cry1Ab + Vip3Aa20 + Cry1A.105 + Cry2Ab2 and Coleopteran-active mCry3A + eCry3.1Ab Insecticidal Protein Mixtures Using *Helicoverpa zea* and *Leptinotarsa decemlineata*
TK0220392	Investigation of the Potential Interaction between Lepidopteran-active Cry1Ab + Vip3Aa20 and Cry1A.105 + Cry2Ab2 Insecticidal Protein Mixtures Using Corn Earworm (*Helicoverpa zea*)
C1616	Quantitation of Endogenous Lipid Transfer Protein (LTP) Allergen in Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 Maize using Aqua™-MRM Mass Spectrometry

Appendix B – Protein expression data

Mean, standard deviation and range of protein levels (μg/g dry weight) from maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 (not treated) and MON 89034, 1507, MIR162, NK603, DAS-40278-9 (not treated), from field trials performed across three locations in the USA in 2015 (n = 15).[a]

Protein	Event(s)	Leaf (V6)	Leaf (R1)	Root (V6)	Root (R1)	Whole plant (V6)	Whole plant (R1)	Pollen (R1)	Grain (R6)	Grain (senescence)
Cry1Ab	**Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21**	207[b] ± 46.4[c] (142–296)[d]	98.2 ± 29.91 (39.6–137)	55.7 ± 8.87 (38.6–69.7)	52.2 ± 12.85 (25.1–71.9)	171 ± 20.9 (142–209)	58.2 ± 23.51 (25.3–97.6)	0.15 ± 0.02 (0.11–0.19)	3.98 ± 0.53 (3.35–5.16)	3.45 ± 0.26 (2.87–3.76)
	Bt11	216.0 ± 44.9 (127–272)	109.0 ± 54.5 (44–210)	57.9 ± 9.85 (35.9–79)	55.3 ± 14.71 (26.1–75)	167 ± 27.7 (121–242)	59.2 ± 27.94 (18.3–90.3)	0.13 ± 0.02 (0.10–0.17)	4.28 ± 0.27 (3.84–4.84)	3.68 ± 0.40 (2.90–4.58)
PAT	**Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21**	0.60 ± 0.16 (0.39–1.03)	0.66 ± 0.13 (0.52–0.96)	1.15 ± 0.45 (0.39–1.86)	0.90 ± 0.28 (< LOQ–1.21)[f]	0.85 ± 0.23 (0.67–1.40)	0.69 ± 0.18 (0.44–1.01)	< LOD[g]	0.04 ± 0.005 (< LOD–0.04)[h]	< LOD[g]
	Bt11	0.71 ± 0.15 (0.49–0.10)	0.75 ± 0.09 (0.56–0.88)	1.74 ± 0.54 (1.14–2.91)	1.01 ± 0.45 (0.17–1.60)	1.04 ± 0.25 (0.62–1.51)	0.86 ± 0.12 (0.66–1.09)	< LOD[g]	0.05 ± 0.01 (< LOQ–0.07)[f]	0.04 ± 0.009 (< LOD–0.05)[h]
Vip3Aa20	**Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21**	173 ± 25.9 (134–225)	123 ± 18.8 (92.9–162)	73.3 ± 19.9 (50.6–105)	28 ± 9.51 (9.69–39.7)	145 ± 32.1 (99–192)	75.5 ± 11.88 (55.3–99.6)	57.9 ± 8.04 (41.7–73.2)	63.1 ± 10.43 (48.7–88.3)	54.8 ± 16.5 (33.6–84)
	MIR162	144 ± 9.60 (129–156)	113 ± 15 (91.7–141)	70.2 ± 11.65 (48.3–93.5)	30.6 ± 11.47 (10.4–53.4)	129 ± 21.8 (91.6–156)	86.3 ± 27.33 (51.3–141)	60.1 ± 7.05 (46.8–70.7)	65.8 ± 13.33 (46.9–91.6)	54.8 ± 12.8 (34.7–77.3)
PMI[e]	**Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21**	36.9 ± 6.56 (28.9–54.6)	27.9 ± 6.26 (17.5–44.9)	17.7 ± 4.63 (7.96–25.2)	11.0 ± 3.21 (4.51–15.9)	30.7 ± 6.36 (22.7–49.3)	17.5 ± 5.59 (11.2–31.6)	91.3 ± 11.14 (82.5–127)	5.76 ± 0.66 (4.85–7.00)	5.02 ± 1.11 (3.43–6.93)
	MIR162	9.48 ± 2.23 (6.04–14.3)	7.92 ± 0.88 (6.14–9.07)	5.04 ± 1.02 (3.60–7.20)	2.77 ± 0.76 (1.45–4.47)	7.72 ± 1.70 (5.15–11.14)	5.86 ± 1.14 (4.04–7.89)	2.73 ± 0.33 (2.25–3.30)	2.36 ± 0.49 (1.78–3.62)	1.54 ± 0.30 (1.14–2.23)
	MIR604	14.5 ± 2.86 (11.3–19.6)	9.76 ± 1.77 (6.59–12.8)	9.00 ± 2.56 (6.43–15.5)	5.05 ± 1.18 (3.17–6.81)	13.0 ± 2.22 (9.93–16.9)	8.18 ± 1.27 (6.10–10.4)	53.0 ± 8.63 (38.0–71.2)	3.33 ± 0.27 (2.68–3.78)	2.18 ± 0.43 (1.58–3.26)
	5307	3.99 ± 0.62 (3.17–4.92)	4.51 ± 0.56 (3.13–5.52)	4.04 ± 1.15 (2.40–6.53)	2.47 ± 0.49 (1.35–3.12)	4.21 ± 0.59 (3.19–5.40)	4.50 ± 0.75 (3.54–6.01)	58.8 ± 12.13 (42.1–80.1)	2.34 ± 0.47 (1.63–3.09)	1.38 ± 0.26 (0.96–1.85)

Protein	Event(s)	Leaf (V6)	Leaf (R1)	Root (V6)	Root (R1)	Whole plant (V6)	Whole plant (R1)	Pollen (R1)	Grain (R6)	Grain (senescence)
mCry3A	**Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21**	16.0 ± 4.16 (8.80–23.7)	13.1 ± 2.12 (9.43–16.3)	26.0 ± 10.55 (11.2–48.2)	13.5 ± 6.83 (6.58–23.8)	12.4 ± 2.86 (8.02–16.8)	10.6 ± 3.22 (6.07–16.2)	0.18 ± 0.03 (0.15–0.23)	0.32 ± 0.09 (0.17–0.49)	0.32 ± 0.12 (0.13–0.56)
	MIR604	16.4 ± 4.23 (9.48–22.0)	13.9 ± 1.37 (11.2–16.3)	25.2 ± 12.21 (10.3–49.0)	14.4 ± 6.87 (6.02–26.3)	12.5 ± 2.19 (7.95–15.6)	12.9 ± 3.15 (8.85–17.7)	0.11 ± 0.02 (0.08–0.14)	0.72 ± 0.08 (0.59–0.91)	0.45 ± 0.099 (0.30–0.70)
Cry1A.105	**Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21**	44.0 ± 15.18 (22.2–71.1)	21.1 ± 6.06 (10.2–32.5)	18.2 ± 3.8 (9.36–24.2)	12.7 ± 2.31 (6.64–16.3)	34.0 ± 10.52 (18.4–50.9)	15.8 ± 4.93 (6.70–24.3)	2.34 ± 0.91 (1.07–3.66)	1.04 ± 0.09 (0.85–1.19)	0.81 ± 0.22 (0.51–1.21)
	MON 89034	43.0 ± 15.58 (15.2–58.6)	27.1 ± 7.10 (19.1–40.1)	19.5 ± 3.57 (14.3–28.8)	14.1 ± 2.1 (10.6–18.0)	33.6 ± 9.53 (20.0–50.9)	22.4 ± 6.06 (15.7–35.8)	2.79 ± 0.44 (1.67–3.47)	1.20 ± 0.27 (0.92–2.04)	0.86 ± 0.20 (0.67–1.23)
Cry2Ab2	**Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21**	89.8 ± 28.9 (54.6–136)	56.3 ± 23.02 (29.0–101)	37.9 ± 13.07 (20.1–65.8)	21.1 ± 7.98 (5.60–31.5)	69.0 ± 21.47 (42.6–104)	43.3 ± 19.28 (13.2–79.9)	< LOQ[i]	2.03 ± 0.48 (1.37–2.81)	0.95 ± 0.22 (0.66–1.37)
	MON 89034	77.4 ± 29.9 (31.1–120)	61.8 ± 18.45 (27.0–83.4)	36.5 ± 14.08 (15.1–58.5)	22.8 ± 6.78 (13.5–33.3)	60.3 ± 24.51 (28.5–101)	46.4 ± 7.59 (33.0–61.4)	< LOQ[i]	2.39 ± 0.46 (1.67–3.09)	1.14 ± 0.28 (0.75–1.67)
eCry3.1Ab	**Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21**	182 ± 90.6 (77.2–339)	54.6 ± 17.23 (24.8–84.6)	48.9 ± 23.3 (16.0–80.6)	9.50 ± 5.01 (< LOQ–16.7)[f]	142 ± 54.9 (66.0–239)	27.9 ± 13.07 (8.75–46.3)	< LOD[g]	2.49 ± 0.36 (1.96–3.11)	2.64 ± 1.14 (1.47–5.37)
	5307	184 ± 73.4 (94.6–309)	64.5 ± 17.3 (39.5–94.5)	53.0 ± 19.68 (24.7–89.7)	8.24 ± 4.99 (1.77–19.4)	140 ± 52.3 (92.0–288)	43.1 ± 8.27 (33.4–61.4)	< LOD[g]	4.14 ± 0.86 (2.68–6.01)	2.93 ± 0.46 (2.25–3.79)
mEPSPS	**Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21**	89.7 ± 32.72 (36.7–144)	64.7 ± 38.24 (26.2–137)	27.8 ± 6.44 (< LOQ–37.8)[f]	25.5 ± 5.54 (< LOQ–38.8)[f]	74.6 ± 34.94 (36.5–132)	51.9 ± 17.81 (22.7–89.7)	207 ± 38.9 (158–281)	12.8 ± 2.74 (8.21–19.1)	8.52 ± 2.03 (< LOQ–11.9)[f]
	GA21	89.1 ± 38.52 (32.7–142)	70.9 ± 34.4 (25.4–124)	35.0 ± 14.27 (< LOQ–60.2)[f]	26.5 ± 6.76 (< LOQ–45.2)[f]	70.3 ± 39.65 (24.5–134)	60.7 ± 25.83 (23.9–100)	152 ± 29.7 (107–201)	15.6 ± 1.79 (13.4–19.7)	9.93 ± 2.20 (< LOQ–13.2)[f]

(a): Number of samples is n = 15 except for: n = 14 for all proteins in pollen of Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21, for Vip3Aa20 and PMI in grain (senescence) of MIR162.
(b): Mean.
(c): Standard deviation.
(d): Range.

(e): PMI levels in maize Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 are a sum of two protein variants; one expressed in MIR162 and 5307 and another expressed in MIR604. These two PMI variants differ by two amino acids.
(f): LOQ (limit of quantification) for PAT is 0.063 μg/g dw in root (R1) of Bt11 × MIR162 × MIR604 × MON 89034 × 5307 × GA21 and 0.031 μg/g dw in grain (senescence) of Bt11; LOQ for Cry2Ab2 = 0.438 μg/g dw in pollen (R1); LOQ for eCry3.1Ab = 1.00 μg/g dw in root (R1); LOQ for mEPSPS = 12.8 μg/g dw in root (V6) and root (R1) and 4.0 μg/g dw in grain (senescence)
(g): < LOD = all samples below the limit of detection.
(h): LOD (limit of detection) for PAT = 0.025 μg/g dw in pollen (R1) and grain (R6); LOD for eCry3.1Ab = 0.13 μg/g dw in pollen (R1).
(i): < LOQ = all samples were below the limit of quantification.

Appendix C – Statistical analysis and statistically significant findings in the 90-day toxicity study in rats on maize 5307

C.1. Statistical analysis of the 90-day study on maize 5307 in rats

The following endpoints were statistically analysed: body weight, body weight gain, food consumption and food utilisation, haematology and coagulation data, clinical chemistry endpoints, absolute (adjusted) and relative organ weights, neurotoxicology endpoints and motion activity data. For all continuous endpoints, the applicant reported mean, standard deviation in terms of the standardised effect sizes (SES) of each dose group for each sex, variable and period or time interval. In the main statistical analysis, for each of the two inclusion rates, rats consuming the test diet were compared with those consuming the respective control diet. For continuous endpoints, a multi-way analysis of variance (ANOVA; factors: treatment, sex and treatment-by-sex interaction) was performed separately for each parameter and period; for organ weight endpoints, terminal body weight was included as a covariate. For food consumption and food utilisation data (with cage-based observations) the cage was considered as the statistical unit in the ANOVA. For each of the other parameters (with individual-based observations), depending on the results of a preliminary 'cage effect' analysis, the ANOVA was applied to either individual animal data (if the cage effect was not significant) or the mean values per cage (if the cage effect was significant). For all the models, in case the sex-by-treatment interaction in the ANOVA was significant (and in any case for sex-specific parameters), a sex-specific analysis was performed. For categorical endpoints (histopathology data), the test and control groups were compared with Fisher's exact test. Historical control data were provided for food consumption and food utilisation and used to assess statistical differences identified for such parameters in the study. Missing data were considered by the Panel and found not impacting the results. A list of statistically significant findings is provided in Table C.1.

Table C.1: Statistically significant findings in 90-day study on maize 5307 in rats

Statistically significant parameter/ endpoint	Finding	GMO Panel interpretation
Body wt	Higher (10%)[(a)] in low dose females d0-28	Due to a higher body weight at the start of dosing (day 0). Not related to treatment.
Body wt gain	Lower (11%) in top dose males d0-14	No effect on terminal body weights. Within normal variation. Not an adverse effect of treatment.
Food consumption (at various time points in the study)	Increased (< 20%) in low dose males and females and high dose females. Decreased in high dose males	No consistent pattern across the groups. Not related to body weight. Within normal variation. Not an adverse effect of treatment.
Food utilisation	Reduced (< 10%) in both male groups weeks 1–13	No impact on terminal body weight. Within normal variation. Not an adverse effect of treatment.
Motor activity	Reduced (< 20%) in top dose males (1–5 & 1–10 mins) and low dose females (21–25 mins). Increased (< 20%) in top dose females.	No consistent pattern. Overall activity counts not significantly changed. Within normal variation. Not an adverse effect of treatment.
Foot splay	Increased (25%) in low dose females.	Not seen in the top dose females. Within normal variation. Not an adverse effect of treatment.
Haemoglobin	Increased (5%) in low dose males.	Low magnitude. Not seen in the top dose males. Within normal variation. Not an adverse effect of treatment.
APTT	Reduced (20%) in top dose males	Within normal variation. Not an adverse effect of treatment.

Statistically significant parameter/ endpoint	Finding	GMO Panel interpretation
MCHC	Reduced (2%) in top dose females.	Small magnitude. No changes in related parameters. Not an adverse effect of treatment.
Prothrombin time	Reduced (6%) in low dose females.	Small magnitude. Not seen in top dose females. Not an adverse effect of treatment.
Albumin:Globulin ratio	Reduced (10%) in low dose males.	Small magnitude. Not seen in top dose males. Not an adverse effect of treatment.
Total protein and albumin	Reduced (< 10%) in top dose females.	Small magnitude. Within normal variation. Not an adverse effect of treatment.
Chloride	Reduced (1%) in low dose males	Small magnitude. Not seen in top dose males. Not an adverse effect of treatment.
Creatinine	Reduced in top dose males (6%) and low dose females (12%)	Reductions are not adverse in isolation. Small magnitude. Within normal variation. Not an adverse effect of treatment.
Spleen weight (absolute)	Reduced (10%) in low dose males	Small magnitude. Within normal variation. Not seen in top dose males. No associated histopathology or haematology findings. Not an adverse effect of treatment.
Spleen weight (relative to body weight)	Reduced (10%) in both male groups	Small magnitude. Within normal variation. No associated histopathology or haematology findings. Not an adverse effect of treatment.
Thymus weight (absolute)	Increased (15%) in top dose females	Small magnitude. Within normal variation. No associated histopathology or haematology findings. Not an adverse effect of treatment.

(a): Where changes are given as percentages (e.g. reduced (30%)) this indicates the magnitude of the change relative to the control value (e.g. 30% means a value of 7 in test group animals versus 10 in controls).

Appendix D – Animal dietary exposure

Table D.1: Derived NEP concentrations for maize products and maize stover

Feed material	Protein content (%)	Reference	Conversion factor for protein content
Grain[(a)]	9.4	Heuzé et al. (2017a)	–
Gluten feed[(b)]	20	Kulp (2000)	2.13
Gluten meal[(b)]	60	Kulp (2000)	6.38
Hominy meal	11.1	Stock et al. (1999)	1.18
Milled by-products[(c)]	8.4	Kulp (2000)	0.894
Forage[(a)]	7.9	Heuzé et al. (2017b)	–
Stover	6.8	Heuzé et al. (2019)	0.861

(a): Measured protein concentrations in grain and in forage (see also Appendix B) are included here for clarity of derivations; all values reported in the table were rounded to three significant figures. The full unrounded values were used during calculation.

(b): The GMO Panel notes that protein content for gluten feed and gluten meal as reported in Kulp (2000) refers to as fed (i.e.: 10% of moisture).

(c): The applicant did not provide a definition of milled by-products for feed use; they refer to coarse grits, meal and flour which, according to Kulp (2000), are dry milling products. In particular for the estimation of ADE, the consumption of coarse grits (protein content 8.4%) was considered by the applicant, as the by-product with the highest protein content (i.e.: 6.6% for flour and 7.2% for meal). However, the GMO Panel considers this approach not appropriate for animal feeding, because milled by products for feed use could include many more ingredients with different and higher protein content.

Table D.2: Dietary exposure to Cry1Ab, Vip3Aa20, mCry3A, Cry1A.105, Cry2Ab2, eCry3.1Ab, mEPSPS, PAT and PMI proteins (mg/kg bw per day) in selected animals, based on the consumption of maize products

Animal species	Feed material	IR%	Cry1A.105
BW (kg)/total diet intake (kg dw)			Dietary exposure (μg/kg bw per day)
Beef cattle 500/12	Gluten Meal	15	0.219
	Forage/Silage	80	0.128
	Grain	80	0.0833
	Gluten Feed	30	0.146
	Hominy Meal	0	0
	Milled Byprods.	30	0.0289
	Stover	25	0.0166
Dairy cattle 650/25	Gluten Meal	20	0.469
	Forage/Silage	60	0.153
	Grain	30	0.0501
	Gluten Feed	30	0.234
	Gluten Meal	20	0.469
	Hominy Meal	0	0
	Milled Byprods.	30	0.0463
	Stover	20	0.0212
Rams/Ewes 75/2.5	Gluten Meal	30	0.61
	Grain	30	0.0434
	Forage/Silage	0	0
	Gluten Feed	30	0.203
	Hominy Meal	0	0
	Milled Byprods.	30	0.0402
	Stover	0	0

Animal species	Feed material	IR%	Cry1A.105
Lambs 40/1.7	Gluten Meal	30	0.777
	Forage/Silage	30	0.0848
	Grain	30	0.0553
	Gluten Feed	30	0.259
	Hominy Meal	0	0
	Milled Byprods.	30	0.0512
	Stover	0	0
Breeding Swine 260/6	Gluten Meal	10	0.141
	Grain	70	0.0701
	Forage/Silage	20	0.0307
	Gluten Feed	20	0.0938
	Hominy Meal	0	0
	Milled Byprods.	75	0.0695
	Stover	20	0.0127
Finishing Swine 100/3	Gluten Meal	10	0.183
	Grain	70	0.0912
	Forage/Silage	0	0
	Gluten Feed	20	0.122
	Hominy Meal	0	0
	Milled Byprods.	75	0.0904
	Stover	0	0
Broiler Hens 1.7/0.12	Gluten Meal	10	0.43
	Grain	70	0.214
	Forage/Silage	0	0
	Gluten Feed	10	0.143
	Hominy Meal	0	0
	Milled Byprods.	60	0.17
	Stover	0	0
Layer Hens 1.9/0.13	Gluten Meal	10	0.417
	Grain	70	0.208
	Forage/Silage	10	0.0455
	Gluten Feed	0	0
	Hominy Meal	20	0.0701
	Milled Byprods.	50	0.137
	Stover	0	0
Turkey 7/0.5	Gluten Meal	10	0.435
	Grain	50	0.155
	Forage/Silage	0	0
	Gluten Feed	0	0
	Hominy Meal	20	0.0732
	Milled Byprods.	50	0.143
	Stover	0	0

Animal species	Feed material	IR%	Cry1Ab
BW (kg)/ total diet intake (kg dw)			Dietary exposure (μg/kg bw per day)
Beef cattle 500/12	Forage/Silage	80	0.333
	Gluten Meal	15	0.213
	Grain	80	0.0809
	Gluten Feed	30	0.142

Animal species	Feed material	IR%	Cry1Ab
	Hominy Meal	0	0
	Milled Byprods.	30	0.0281
	Stover	25	0.0432
Dairy cattle 650/25	Gluten Meal	20	0.455
	Forage/Silage	60	0.4
	Grain	30	0.0486
	Gluten Feed	30	0.228
	Hominy Meal	0	0
	Milled Byprods.	30	0.045
	Stover	20	0.0553
Rams/Ewes 75/2.5	Gluten Meal	30	0.592
	Grain	30	0.0422
	Forage/Silage	0	0
	Gluten Feed	30	0.197
	Hominy Meal	0	0
	Milled Byprods.	30	0.039
	Stover	0	0
Lambs 40/1.7	Gluten Meal	30	0.755
	Forage/Silage	30	0.221
	Grain	30	0.0538
	Gluten Feed	30	0.252
	Hominy Meal	0	0
	Milled Byprods.	30	0.0497
	Stover	0	0
Breeding Swine 260/6	Gluten Meal	10	0.137
	Forage/Silage	20	0.08
	Grain	70	0.0681
	Gluten Feed	20	0.0911
	Hominy Meal	0	0
	Milled Byprods.	75	0.0675
	Stover	20	0.0332
Finishing Swine 100/3	Gluten Meal	10	0.178
	Grain	70	0.0885
	Forage/Silage	0	0
	Gluten Feed	20	0.118
	Hominy Meal	0	0
	Milled Byprods.	75	0.0878
	Stover	0	0
Broiler Hens 1.7/0.12	Gluten Meal	10	0.418
	Grain	70	0.208
	Forage/Silage	0	0
	Gluten Feed	10	0.139
	Hominy Meal	0	0
	Milled Byprods.	60	0.165
	Stover	0	0
Layer Hens 1.9/0.13	Gluten Meal	10	0.405
	Grain	70	0.202
	Forage/Silage	10	0.119
	Gluten Feed	0	0
	Hominy Meal	20	0.0681

Animal species	Feed material	IR%	Cry1Ab
	Milled Byprods.	50	0.133
	Stover	0	0
Turkey 7/0.5	Gluten Meal	10	0.423
	Grain	50	0.151
	Forage/Silage	0	0
	Gluten Feed	0	0
	Hominy Meal	20	0.0711
	Milled Byprods.	50	0.139
	Stover	0	0

Animal species	Feed material	IR%	Cry2Ab2
BW (kg)/ total diet intake (kg dw)			Dietary exposure (μg/kg bw per day)
Beef cattle 500/12	Forage/Silage	80	0.362
	Gluten Meal	15	0.0867
	Grain	80	0.0329
	Gluten Feed	30	0.0578
	Hominy Meal	0	0
	Milled Byprods.	30	0.0114
	Stover	25	0.0469
Dairy cattle 650/25	Forage/Silage	60	0.435
	Gluten Meal	20	0.185
	Grain	30	0.0198
	Gluten Feed	30	0.0927
	Hominy Meal	0	0
	Milled Byprods.	30	0.0183
	Stover	20	0.0601
Rams/Ewes 75/2.5	Gluten Meal	30	0.241
	Grain	30	0.0172
	Forage/Silage	0	0
	Gluten Feed	30	0.0803
	Hominy Meal	0	0
	Milled Byprods.	30	0.0159
	Stover	0	0
Lambs 40/1.7	Gluten Meal	30	0.307
	Forage/Silage	30	0.24
	Grain	30	0.0219
	Gluten Feed	30	0.102
	Hominy Meal	0	0
	Milled Byprods.	30	0.0202
	Stover	0	0
Breeding Swine 260/6	Forage/Silage	20	0.087
	Gluten Meal	10	0.0556
	Grain	70	0.0277
	Gluten Feed	20	0.0371
	Hominy Meal	0	0
	Milled Byprods.	75	0.0275
	Stover	20	0.0361
Finishing Swine 100/3	Gluten Meal	10	0.0723
	Grain	70	0.036

Animal species	Feed material	IR%	Cry2Ab2
	Forage/Silage	0	0
	Gluten Feed	20	0.0482
	Hominy Meal	0	0
	Milled Byprods.	75	0.0357
	Stover	0	0
Broiler Hens 1.7/0.12	Gluten Meal	10	0.17
	Grain	70	0.0848
	Forage/Silage	0	0
	Gluten Feed	10	0.0567
	Hominy Meal	0	0
	Milled Byprods.	60	0.0672
	Stover	0	0
Layer Hens 1.9/0.13	Gluten Meal	10	0.165
	Forage/Silage	10	0.129
	Grain	70	0.0822
	Gluten Feed	0	0
	Hominy Meal	20	0.0277
	Milled Byprods.	50	0.0543
	Stover	0	0
Turkey 7/0.5	Gluten Meal	10	0.172
	Grain	50	0.0613
	Forage/Silage	0	0
	Gluten Feed	0	0
	Hominy Meal	20	0.0289
	Milled Byprods.	50	0.0567
	Stover	0	0

Animal species	Feed material	IR%	eCry3.1Ab
BW (kg)/ total diet intake (kg dw)			Dietary exposure (μg/kg bw per day)
Beef cattle 500/12	Forage/Silage	80	0.125
	Gluten Meal	15	0.103
	Grain	80	0.0393
	Gluten Feed	30	0.0689
	Hominy Meal	0	0
	Milled Byprods.	30	0.0136
	Stover	25	0.0162
Dairy cattle 650/25	Gluten Meal	20	0.221
	Forage/Silage	60	0.15
	Grain	30	0.0236
	Gluten Feed	30	0.11
	Hominy Meal	0	0
	Milled Byprods.	30	0.0218
	Stover	20	0.0208
Rams/Ewes 75/2.5	Gluten Meal	30	0.287
	Grain	30	0.0205
	Forage/Silage	0	0
	Gluten Feed	30	0.0957
	Hominy Meal	0	0
	Milled Byprods.	30	0.0189
	Stover	0	0

Animal species	Feed material	IR%	eCry3.1Ab
Lambs 40/1.7	Gluten Meal	30	0.366
	Forage/Silage	30	0.0829
	Grain	30	0.0261
	Gluten Feed	30	0.122
	Hominy Meal	0	0
	Milled Byprods.	30	0.0241
	Stover	0	0
Breeding Swine 260/6	Gluten Meal	10	0.0663
	Grain	70	0.033
	Forage/Silage	20	0.03
	Gluten Feed	20	0.0442
	Hominy Meal	0	0
	Milled Byprods.	75	0.0328
	Stover	20	0.0125
Finishing Swine 100/3	Gluten Meal	10	0.0862
	Grain	70	0.043
	Forage/Silage	0	0
	Gluten Feed	20	0.0574
	Hominy Meal	0	0
	Milled Byprods.	75	0.0426
	Stover	0	0
Broiler Hens 1.7/0.12	Gluten Meal	10	0.203
	Grain	70	0.101
	Forage/Silage	0	0
	Gluten Feed	10	0.0676
	Hominy Meal	0	0
	Milled Byprods.	60	0.0801
	Stover	0	0
Layer Hens 1.9/0.13	Gluten Meal	10	0.197
	Grain	70	0.098
	Forage/Silage	10	0.0445
	Gluten Feed	0	0
	Hominy Meal	20	0.0331
	Milled Byprods.	50	0.0647
	Stover	0	0
Turkey 7/0.5	Gluten Meal	10	0.205
	Grain	50	0.0731
	Forage/Silage	0	0
	Gluten Feed	0	0
	Hominy Meal	20	0.0345
	Milled Byprods.	50	0.0676
	Stover	0	0

Animal species	Feed material	IR%	mCry3A
BW (kg)/ total diet intake (kg dw)			Dietary exposure (μg/kg bw per day)
Beef cattle 500/12	Forage/Silage	80	0.0528
	Gluten Meal	15	0.0111
	Grain	80	0.00421
	Gluten Feed	30	0.00739

Animal species	Feed material	IR%	mCry3A
	Hominy Meal	0	0
	Milled Byprods.	30	0.00146
	Stover	25	0.00685
Dairy cattle 650/25	Forage/Silage	60	0.0635
	Gluten Meal	20	0.0237
	Grain	30	0.00253
	Gluten Feed	30	0.0118
	Hominy Meal	0	0
	Milled Byprods.	30	0.00234
	Stover	20	0.00878
Rams/Ewes 75/2.5	Gluten Meal	30	0.0308
	Grain	30	0.00219
	Forage/Silage	0	0
	Gluten Feed	30	0.0103
	Hominy Meal	0	0
	Milled Byprods.	30	0.00203
	Stover	0	0
Lambs 40/1.7	Gluten Meal	30	0.0393
	Forage/Silage	30	0.0351
	Grain	30	0.0028
	Gluten Feed	30	0.0131
	Hominy Meal	0	0
	Milled Byprods.	30	0.00259
	Stover	0	0
Breeding Swine 260/6	Forage/Silage	20	0.0127
	Gluten Meal	10	0.00711
	Grain	70	0.00354
	Gluten Feed	20	0.00474
	Hominy Meal	0	0
	Milled Byprods.	75	0.00351
	Stover	20	0.00527
Finishing Swine 100/3	Gluten Meal	10	0.00924
	Grain	70	0.00461
	Forage/Silage	0	0
	Gluten Feed	20	0.00616
	Hominy Meal	0	0
	Milled Byprods.	75	0.00457
	Stover	0	0
Broiler Hens 1.7/0.12	Gluten Meal	10	0.0217
	Grain	70	0.0108
	Forage/Silage	0	0
	Gluten Feed	10	0.00725
	Hominy Meal	0	0
	Milled Byprods.	60	0.00859
	Stover	0	0
Layer Hens 1.9/0.13	Gluten Meal	10	0.0211
	Forage/Silage	10	0.0188
	Grain	70	0.0105
	Gluten Feed	0	0
	Hominy Meal	20	0.00354

Animal species	Feed material	IR%	mCry3A
	Milled Byprods.	50	0.00694
	Stover	0	0
Turkey 7/0.5	Gluten Meal	10	0.022
	Grain	50	0.00783
	Forage/Silage	0	0
	Gluten Feed	0	0
	Hominy Meal	20	0.0037
	Milled Byprods.	50	0.00725
	Stover	0	0

Animal species	Feed material	IR%	mEPSPS
BW (kg)/ total diet intake (kg dw)			Dietary exposure (μg/kg bw per day)
Beef cattle 500/12	Gluten Meal	15	0.455
	Forage/Silage	80	0.292
	Grain	80	0.173
	Gluten Feed	30	0.303
	Hominy Meal	0	0
	Milled Byprods.	30	0.06
	Stover	25	0.0379
Dairy cattle 650/25	Gluten Meal	20	0.972
	Forage/Silage	60	0.351
	Grain	30	0.104
	Gluten Feed	30	0.486
	Hominy Meal	0	0
	Milled Byprods.	30	0.0961
	Stover	20	0.0486
Rams/Ewes 75/2.5	Gluten Meal	30	1.26
	Grain	30	0.09
	Forage/Silage	0	0
	Gluten Feed	30	0.421
	Hominy Meal	0	0
	Milled Byprods.	30	0.0833
	Stover	0	0
Lambs 40/1.7	Gluten Meal	30	1.61
	Forage/Silage	30	0.194
	Grain	30	0.115
	Gluten Feed	30	0.537
	Hominy Meal	0	0
	Milled Byprods.	30	0.106
	Stover	0	0
Breeding Swine 260/6	Gluten Meal	10	0.292
	Grain	70	0.145
	Forage/Silage	20	0.0703
	Gluten Feed	20	0.194
	Hominy Meal	0	0
	Milled Byprods.	75	0.144
	Stover	20	0.0291

Animal species	Feed material	IR%	mEPSPS
Finishing Swine 100/3	Gluten Meal	10	0.379
	Grain	70	0.189
	Forage/Silage	0	0
	Gluten Feed	20	0.253
	Hominy Meal	0	0
	Milled Byprods.	75	0.187
	Stover	0	0
Broiler Hens 1.7/0.12	Gluten Meal	10	0.892
	Grain	70	0.445
	Forage/Silage	0	0
	Gluten Feed	10	0.297
	Hominy Meal	0	0
	Milled Byprods.	60	0.353
	Stover	0	0
Layer Hens 1.9/0.13	Gluten Meal	10	0.865
	Grain	70	0.431
	Forage/Silage	10	0.104
	Gluten Feed	0	0
	Hominy Meal	20	0.145
	Milled Byprods.	50	0.285
	Stover	0	0
Turkey 7/0.5	Gluten Meal	10	0.903
	Grain	50	0.321
	Forage/Silage	0	0
	Gluten Feed	0	0
	Hominy Meal	20	0.152
	Milled Byprods.	50	0.297
	Stover	0	0

Animal species	Feed material	IR%	PAT
BW (kg)/ total diet intake (kg dw)			Dietary exposure (μg/kg bw per day)
Beef cattle 500/12	Forage/Silage	80	0.00383
	Gluten Meal	15	0.00109
	Grain	80	0.000412
	Gluten Feed	30	0.000724
	Hominy Meal	0	0
	Milled Byprods.	30	0.000143
	Stover	25	0.000496
Dairy cattle 650/25	Forage/Silage	60	0.0046
	Gluten Meal	20	0.00232
	Grain	30	0.000248
	Gluten Feed	30	0.00116
	Hominy Meal	0	0
	Milled Byprods.	30	0.000229
	Stover	20	0.000636
Rams/Ewes 75/2.5	Gluten Meal	30	0.00302
	Grain	30	0.000215
	Forage/Silage	0	0
	Gluten Feed	30	0.00101

Animal species	Feed material	IR%	PAT
	Hominy Meal	0	0
	Milled Byprods.	30	0.000199
	Stover	0	0
Lambs 40/1.7	Gluten Meal	30	0.00385
	Forage/Silage	30	0.00254
	Grain	30	0.000274
	Gluten Feed	30	0.00128
	Hominy Meal	0	0
	Milled Byprods.	30	0.000253
	Stover	0	0
Breeding Swine 260/6	Forage/Silage	20	0.00092
	Gluten Meal	10	0.000696
	Grain	70	0.000347
	Gluten Feed	20	0.000464
	Hominy Meal	0	0
	Milled Byprods.	75	0.000344
	Stover	20	0.000381
Finishing Swine 100/3	Gluten Meal	10	0.000905
	Grain	70	0.000451
	Forage/Silage	0	0
	Gluten Feed	20	0.000603
	Hominy Meal	0	0
	Milled Byprods.	75	0.000447
	Stover	0	0
Broiler Hens 1.7/0.12	Gluten Meal	10	0.00213
	Grain	70	0.00106
	Forage/Silage	0	0
	Gluten Feed	10	0.00071
	Hominy Meal	0	0
	Milled Byprods.	60	0.000842
	Stover	0	0
Layer Hens 1.9/0.13	Gluten Meal	10	0.00206
	Forage/Silage	10	0.00136
	Grain	70	0.00103
	Gluten Feed	0	0
	Hominy Meal	20	0.000347
	Milled Byprods.	50	0.00068
	Stover	0	0
Turkey 7/0.5	Gluten Meal	10	0.00215
	Grain	50	0.000767
	Forage/Silage	0	0
	Gluten Feed	0	0
	Hominy Meal	20	0.000362
	Milled Byprods.	50	0.00071
	Stover	0	0

Animal species	Feed material	IR%	PMI
BW (kg)/ total diet intake (kg dw)			Dietary exposure (μg/kg bw per day)
Beef cattle 500/12	Gluten Meal	15	0.356
	Forage/Silage	80	0.281
	Grain	80	0.135
	Gluten Feed	30	0.237
	Hominy Meal	0	0
	Milled Byprods.	30	0.0469
	Stover	25	0.0364
Dairy cattle 650/25	Gluten Meal	20	0.76
	Forage/Silage	60	0.338
	Grain	30	0.0812
	Gluten Feed	30	0.38
	Hominy Meal	0	0
	Milled Byprods.	30	0.0751
	Stover	20	0.0467
Rams/Ewes 75/2.5	Gluten Meal	30	0.988
	Grain	30	0.0703
	Forage/Silage	0	0
	Gluten Feed	30	0.329
	Hominy Meal	0	0
	Milled Byprods.	30	0.0651
	Stover	0	0
Lambs 40/1.7	Gluten Meal	30	1.26
	Forage/Silage	30	0.186
	Grain	30	0.0897
	Gluten Feed	30	0.42
	Hominy Meal	0	0
	Milled Byprods.	30	0.083
	Stover	0	0
Breeding Swine 260/6	Gluten Meal	10	0.228
	Grain	70	0.114
	Forage/Silage	20	0.0675
	Gluten Feed	20	0.152
	Hominy Meal	0	0
	Milled Byprods.	75	0.113
	Stover	20	0.028
Finishing Swine 100/3	Gluten Meal	10	0.296
	Grain	70	0.148
	Forage/Silage	0	0
	Gluten Feed	20	0.198
	Hominy Meal	0	0
	Milled Byprods.	75	0.146
	Stover	0	0
Broiler Hens 1.7/0.12	Gluten Meal	10	0.697
	Grain	70	0.348
	Forage/Silage	0	0
	Gluten Feed	10	0.232
	Hominy Meal	0	0
	Milled Byprods.	60	0.276
	Stover	0	0

Animal species	Feed material	IR%	PMI
Layer Hens 1.9/0.13	Gluten Meal	10	0.676
	Grain	70	0.337
	Forage/Silage	10	0.1
	Gluten Feed	0	0
	Hominy Meal	20	0.114
	Milled Byprods.	50	0.223
	Stover	0	0
Turkey 7/0.5	Gluten Meal	10	0.706
	Grain	50	0.251
	Forage/Silage	0	0
	Gluten Feed	0	0
	Hominy Meal	20	0.119
	Milled Byprods.	50	0.232
	Stover	0	0

Animal species	Feed material	IR%	Vip3Aa20
BW (kg)/ total diet intake (kg dw)			Dietary exposure (μg/kg bw per day)
Beef cattle 500/12	Gluten Meal	15	2.4
	Forage/Silage	80	1.24
	Grain	80	0.912
	Gluten Feed	30	1.6
	Hominy Meal	0	0
	Milled Byprods.	30	0.316
	Stover	25	0.16
Dairy cattle 650/25	Gluten Meal	20	5.13
	Forage/Silage	60	1.49
	Grain	30	0.548
	Gluten Feed	30	2.57
	Hominy Meal	0	0
	Milled Byprods.	30	0.507
	Stover	20	0.206
Rams/Ewes 75/2.5	Gluten Meal	30	6.67
	Grain	30	0.475
	Forage/Silage	0	0
	Gluten Feed	30	2.22
	Hominy Meal	0	0
	Milled Byprods.	30	0.439
	Stover	0	0
Lambs 40/1.7	Gluten Meal	30	8.5
	Forage/Silage	30	0.822
	Grain	30	0.606
	Gluten Feed	30	2.83
	Hominy Meal	0	0
	Milled Byprods.	30	0.56
	Stover	0	0
Breeding Swine 260/6	Gluten Meal	10	1.54
	Grain	70	0.767
	Forage/Silage	20	0.298
	Gluten Feed	20	1.03

Animal species	Feed material	IR%	Vip3Aa20
	Hominy Meal	0	0
	Milled Byprods.	75	0.761
	Stover	20	0.123
Finishing Swine 100/3	Gluten Meal	10	2
	Grain	70	0.998
	Forage/Silage	0	0
	Gluten Feed	20	1.33
	Hominy Meal	0	0
	Milled Byprods.	75	0.989
	Stover	0	0
Broiler Hens 1.7/0.12	Gluten Meal	10	4.71
	Grain	70	2.35
	Forage/Silage	0	0
	Gluten Feed	10	1.57
	Hominy Meal	0	0
	Milled Byprods.	60	1.86
	Stover	0	0
Layer Hens 1.9/0.13	Gluten Meal	10	4.56
	Grain	70	2.28
	Forage/Silage	10	0.441
	Gluten Feed	0	0
	Hominy Meal	20	0.768
	Milled Byprods.	50	1.5
	Stover	0	0
Turkey 7/0.5	Grain	50	1.7
	Forage/Silage	0	0
	Gluten Feed	0	0
	Hominy Meal	20	0.801
	Milled Byprods.	50	1.57
	Stover	0	0
	Grain	50	1.7