Assessment of genetically modified maize 1507 x MIR162 x MON810 x NK603 and subcombinations, for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2015-127)

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Assessment of genetically modified maize 1507 × MIR162 × MON810 × NK603 and subcombinations, for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2015-127)

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Abstract

Maize 1507 × MIR162 × MON810 × NK603 (four-event stack maize) was produced by conventional crossing to combine four single events: 1507, MIR162, MON810 and NK603. The GMO Panel previously assessed the four single events and six of the subcombinations and did not identify safety concerns. No new data on the single events or the six subcombinations that could lead to modification of the original conclusions on their safety were identified. The molecular characterisation, comparative analysis (agronomic, phenotypic and compositional characteristics) and the outcome of the toxicological, allergenicity and nutritional assessment indicate that the combination of the single maize events and of the newly expressed proteins in the four-event stack maize does not give rise to food and feed safety and nutritional concerns. The GMO Panel concludes that the four-event stack maize, as described in this application, is as safe as its non-GM comparator and the non-GM reference varieties tested. In the case of accidental release of viable seeds of the four-event stack maize into the environment, this would not raise environmental safety concerns. The GMO Panel assessed the likelihood of interactions among the single events in the four maize subcombinations not previously assessed and concludes that these are expected to be as safe as the single events, the previously assessed subcombinations and the four-event stack maize. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of the four-event stack maize. Post-market monitoring of food/feed is not considered necessary. The GMO Panel concludes that the four-event stack maize and its subcombinations are as safe as the non-GM comparator and the tested non-GM reference varieties with respect to potential effects on human and animal health and the environment.

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

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Summary

Following the submission of application EFSA-GMO-NL-2015-127 under Regulation (EC) No 1829/2003 from Pioneer (hereafter referred to as 'the applicant'), the Panel on Genetically Modified Organisms of the European Food Safety Authority (hereafter referred to as the 'GMO Panel') was asked to deliver a scientific opinion on genetically modified (GM) maize 1507 × MIR162 × MON810 × NK603 (referred to hereafter to as 'the four-event stack maize') and its subcombinations independently of their origin, according to the Commission Regulation (EU) No 503/2013 (referred to hereafter as 'subcombinations'). The scope of application EFSA-GMO-NL-2015-127 is for import, processing, and food and feed uses within the European Union (EU) of maize 1507 × MIR162 × MON810 × NK603 and all its subcombinations independently of their origin, and does not include cultivation in the EU.

The term 'subcombination' refers to any combination of up to four of the events present in the four-event stack maize. The safety of subcombinations occurring as segregating progeny in the harvested grains of maize 1507 × MIR162 × MON810 × NK603 is evaluated in the context of the assessment of the four-event stack maize. The safety of subcombinations that have either been or could be produced by conventional crossing through targeted breeding approaches, and which can be bred, produced and marketed independently of the four-event stack, are risk assessed separately in the present scientific opinion.

The four-event stack maize was produced by conventional crossing to combine four single maize events: 1507 (expressing the Cry1F and PAT proteins), MON810 (expressing the Cry1Ab protein), MIR162 (expressing the Vip3Aa20 and PMI proteins) and NK603 (expressing the CP4 EPSPS and CP4 EPSPS L214P proteins) to confer resistance to certain lepidopteran pests and tolerance to glyphosate- and glufosinate ammonium-based herbicides.

The GMO Panel evaluated the four-event stack maize and its subcombinations with reference to the scope and appropriate principles described in its guidelines for the risk assessment of GM plants and derived food and feed, the environmental risk assessment of GM plants and the post-market environmental monitoring (PMEM) of GM plants. The GMO Panel considered the information submitted in application EFSA-GMO-NL-2016-127, additional information provided by the applicant during the risk assessment, the scientific comments submitted by the Member States (MS) and the relevant scientific literature.

The previous assessments of the single events 1507, MON810, MIR162 and NK603 and six of the subcombinations provided a basis for the assessment of the four-event stack maize and the remaining four subcombinations. No safety concerns were identified by the GMO Panel in the previous assessments. No safety issue concerning the four single maize events was identified by the updated bioinformatic analyses, nor reported by the applicant since the publication of the previous GMO Panel scientific opinions. Therefore, the GMO Panel considers that its previous conclusions on the safety of the single maize events remain valid.

For the four-event stack maize, the risk assessment included the molecular characterisation of the inserted DNA and analysis of protein expression. An evaluation of the comparative analysis of agronomic, phenotypic and compositional characteristics was undertaken, and the safety of the newly expressed proteins and the whole food and feed were evaluated with respect to potential toxicity, allergenicity and nutritional characteristics. An evaluation of environmental impacts and the post-market environmental monitoring (PMEM) plan was also undertaken.

The molecular data establish that the events stacked in maize 1507 × MIR162 × MON810 × NK603 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins are similar in the four-event stack maize and in the single events. No indications of interactions that may affect the integrity of the events and the levels of the newly expressed proteins in this four-event stack maize were identified.

The comparative analysis of forage and grain composition and agronomic and phenotypic characteristics identified no differences between maize 1507 × MIR162 × MON810 × NK603 and the non-GM comparator that required further assessment for food/feed safety or environmental impact.

The molecular characterisation, the comparative analysis and the outcome of the toxicological, allergenicity and nutritional assessment indicate that the combination of the single maize events and of the newly expressed proteins in the four-event stack maize does not give rise to food and feed safety and nutritional concerns. The GMO Panel concludes that maize 1507 × MIR162 × MON810 × NK603, as described in this application, is as safe as and nutritionally equivalent to its non-GM comparator and the non-GM reference varieties tested.

Considering the combined events and their potential interactions, the outcome of the comparative analysis, and the routes and levels of exposure, the GMO Panel concludes that maize 1507 × MIR162 × MON810 × NK603 would not raise safety concerns in the case of accidental release of viable GM maize grains into the environment.

Since no new safety concerns were identified for the six 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 four subcombinations included in the scope of application EFSA-GMO-NL-2015-127, no experimental data were provided. The GMO Panel assessed the possibility of interactions among the events in the four subcombinations and concludes that these subcombinations would not raise safety concerns. These subcombinations are therefore expected to be as safe as and nutritionally equivalent to the single events, the previously assessed subcombinations and the four-event stack maize.

Based on the relevant publications identified through the literature searches, the GMO Panel does not identify any safety issue pertaining to the intended uses of maize 1507 × MIR162 × MON810 × NK603 and its subcombinations. In the context of annual PMEM reports, the applicant could further fine-tune future literature searches according to the GMO Panel recommendations given in this scientific opinion.

Given the absence of safety concerns for foods and feeds from maize 1507 × MIR162 × MON810 × NK603 and its subcombinations, the GMO Panel considers that post-market monitoring of these products is not necessary. The PMEM plan and reporting intervals are in line with the intended uses of the four-event stack maize and its subcombinations.

The GMO Panel concludes that maize 1507 × MIR162 × MON810 × NK603 and its subcombinations, as described in this application, are as safe as the non-GM comparator and the tested non-GM reference varieties with respect to potential effects on human and animal health and the environment.

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1. Introduction

The scope of application EFSA-GMO-NL-2015-127 is for food and feed uses, import and processing in the European Union (EU) of the genetically modified (GM) herbicide-tolerant and insect-resistant maize 1507 × MIR162 × MON810 × NK603 and all its subcombinations independently of their origin and does not include cultivation in EU.

1.1. Background

On 18 December 2015, the European Food Safety Authority (EFSA) received from the Competent Authority of The Netherlands application EFSA-GMO-NL-2015-127 for authorisation of maize 1507 × MIR162 × MON810 × NK603 (Unique Identifier DAS-Ø15Ø7-1 × MON-ØØ81Ø-6 × SYN-IR162-4 × MON-ØØ6Ø3-6), submitted by Pioneer Hi-Bred International, Inc. (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003[1].

Following receipt of application EFSA-GMO-NL-2015-127, EFSA informed EU Member States and the European Commission and made the summary of the application available to the public on the EFSA website.[2]

EFSA checked the application for compliance with the relevant requirements of Regulation (EC) No 1829/2003 and Regulation (EU) No 503/2013[3] and, when needed, asked the applicant to supplement the initial application. On 9 February 2016, EFSA declared the application valid and made the application available to the Member States and the European Commission.

From validity date, EFSA and its scientific Panel on Genetically Modified Organisms (hereafter referred to as 'the GMO Panel') endeavoured to respect a time limit of 6 months to issue a scientific opinion on application EFSA-GMO-NL-2015-127. Such time limit was extended whenever EFSA and/or its GMO Panel requested supplementary information to the applicant. According to Regulation (EC) No 1829/2003, any supplementary information provided by the applicant during the risk assessment was made available to the EU Member States and European Commission (for further details, see the section 'Documentation', below).

In accordance with Regulation (EC) No 1829/2003, EFSA consulted the nominated risk assessment bodies of EU Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC[4]. The EU Member States had three months to make their opinion known on application EFSA-GMO-NL-2015-127 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 1507 × MIR162 × MON810 × NK603 and all its subcombinations independently of their origin according to the of application EFSA-GMO-NL-2015-127.

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

In addition to the present scientific opinion, EFSA and its GMO Panel were also asked to report on the particulars listed under Articles 6(5) and 18(5) of Regulation (EC) No 1829/2003. The relevant information is made available in the EFSA Register of Questions,[6] including the information required under Annex II to the Cartagena Protocol; a labelling proposal; a post-market environmental monitoring (PMEM) plan as provided by the applicant; the methods, validated by the Community reference laboratory, for detection, including sampling, identification of the transformation events in the food-feed and/or foods-feeds produced from it and the appropriate reference materials.

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.
[2] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2015-00841
[3] Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorization of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. OJ L157, 8.6.2013, p. 1–48.
[4] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modifed organisms and repealing Council Directive 90/220/EEC. OJ L 106, 12.3.2001, p. 1–38.
[5] Opinions of the nominated risk assessment bodies of EU Member States can be found at the EFSA Register of Questions (http://registerofquestions.efsa.europa.eu/roqFrontend/login), querying the assigned Question Number.
[6] http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2015-00841

2. Data and methodologies

2.1. Data

The GMO Panel based its scientific risk assessment of maize 1507 × MIR162 × MON810 × NK603 on the valid application EFSA-GMO-NL-2015-127, additional information provided by the applicant during the risk assessment, relevant scientific comments submitted by EU Member States and relevant peer-reviewed scientific publications. As part of this comprehensive information package, the GMO Panel received additional unpublished studies submitted by the applicant in order to comply with the specific provisions of Regulation (EU) No 503/2013. A list of these additional unpublished studies is provided in Appendix A.

2.2. Methodologies

The GMO Panel conducted its assessment in line with the principles described in Regulation (EU) No 503/2013, its applicable guidelines (i.e. EFSA GMO Panel, 2010a, 2011a,b, 2015, 2017a) and explanatory notes and statements (i.e. EFSA GMO Panel, 2010b; EFSA, 2014, 2017a,b, 2019) for the risk assessment of GM plants.

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

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

In the frame of the contracts OC/EFSA/GMO/2013/01, OC/EFSA/GMO/2014/01 and OC/EFSA/GMO/2018/02 contractors performed preparatory work and delivered reports on the methods applied by the applicant in performing bioinformatic, statistical and toxicological analyses, respectively.

3. Assessment

3.1. Introduction

Application EFSA-GMO-NL-2015-127 covers the four-event stack maize 1507 × MON810 × MIR162 × NK603 and all its 10 subcombinations independently of their origin (Table 1).

Table 1: Eleven combinations of the events covered by the scope of the application EFSA-GMO-NL-2015-127

Degree of stacking	Events
Four-event stack	MON810 × 1507 × NK603 × MIR162
Three-event stack	1507 × NK603 × MIR162
	MON810 × NK603 × MIR162
	MON810 × 1507 × MIR162
	MON810 × 1507 × NK603
Two-event stack	NK603 × MIR162
	1507 × MIR162
	1507 × NK603
	MON810 × MIR162
	MON810 × NK603
	MON810 × 1507

The term 'subcombination' refers to any combination of up to three of the maize events 1507, MON810, MIR162 and NK603.

The safety of subcombinations occurring as segregating progeny in harvested grains of maize 1507 × MON810 × MIR162 × NK603 is evaluated in the context of the assessment of the four-event stack maize in Section 3.5 of the present scientific opinion.

'Subcombination' also covers combinations that have either been or could be produced by conventional crossing through targeted breeding approaches (EFSA GMO Panel, 2011a). These are

maize stacks that can be bred, produced and marketed independently of the four-event stack maize. These subcombinations are assessed in Section 3.5 of this scientific opinion.

The four-event stack maize was produced by conventional crossing to combine four single maize events: 1507 (expressing the Cry1F and PAT proteins), MON810 (expressing the Cry1Ab protein), MIR162 (expressing the Vip3Aa20 and PMI proteins) and NK603 (expressing the CP4 EPSPS and CP4 EPSPS L214P proteins) to confer resistance to certain lepidopteran pests and tolerance to glyphosate- and glufosinate ammonium-based herbicides. It should be noted that the assessment of herbicide residues in maize herbicide-tolerant crops relevant for this application has been investigated by the EFSA Pesticides Unit (EFSA, 2018a).

All four single maize events, the two-event stacks MON810 × 1507, MON810 × NK603, 1507 × NK603, 1507 × MIR162 and NK603 × MIR162, and the three-event stack MON810 × 1507 × NK603 have been previously assessed by the GMO Panel (see Table 2) and no safety concerns were identified.

Table 2: Single maize events and subcombinations of maize 1507 × MON 810 × MIR162 × NK603 previously assessed by the GMO Panel

Events	Application or mandate	Reference
MON810	EFSA-GMO-RX-MON810	EFSA (2009a)
1507	EFSA-Q-2004-011	EFSA (2004a)
	EFSA-GMO-NL-2004-02	EFSA (2005a)
	EFSA-Q-2006-00330	EFSA (2005b)
	EFSA-GMO-RX-1507	EFSA (2009b)
	EFSA-GMO-RX-001	EFSA GMO Panel (2017b)
NK603	Art4_NK603	EFSA (2004b)
	CE/ES/00/01	EFSA (2007)
	EFSA-GMO-NL-2005-22	EFSA (2009c)
	EFSA-GMO-RX-NK603	EFSA (2009c)
MIR162	EFSA-GMO-DE-2010-82	EFSA GMO Panel (2012)
MON810 × 1507	EFSA-GMO-NL-2011-92	EFSA GMO Panel (2017c)
MON810 × NK603	EFSA-GMO-UK-2004-01	EFSA (2005c)
	C/GB/02/M3/3	EFSA (2005d)
	EFSA-GMO-NL-2011-92	EFSA GMO Panel (2017c)
	EFSA-GMO-RX-007	EFSA GMO Panel (2018a)
1507 × NK603	EFSA-GMO-UK-2004-05	EFSA (2006)
	EFSA-GMO-NL-2009-65	EFSA GMO Panel (2010c)
	M-2011-0066	EFSA GMO Panel (2011c)
	EFSA-GMO-NL-2011-92	EFSA GMO Panel (2017c)
	EFSA-GMO-NL-2013-112	EFSA GMO Panel (2019a)
	EFSA-GMO-RX-008	EFSA GMO Panel (2018b)
1507 × MIR162	EFSA-GMO-DE-2010-86	EFSA GMO Panel (2018c)
	EFSA-GMO-DE-2011-103	EFSA GMO Panel (2019b)
NK603 × MIR162	EFSA-GMO-NL-2016-131	EFSA GMO Panel (2019c)
	EFSA-GMO-NL-2016-134	EFSA GMO Panel (2019d)
MON810 × 1507 × NK603	EFSA-GMO-NL-2011-92	EFSA GMO Panel (2017c)

3.2. Updated information on single events[7]

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

The applicant clarified that the maize 1507 sequence reported in this application is identical to the corrected maize 1507 sequence. The evaluation of the corrected sequencing data and the

[7] Dossier: Part II - Section 1.2.2.2.v and vi; additional information: 8/3/2017 and 16/4/2020; spontaneous information: 17/11/2017, 17/12/2019 and 18/3/2020.

bioinformatic analyses performed on this sequence did not give rise to safety issues (EFSA GMO Panel, 2017b).

Updated bioinformatic analyses for events 1507, MON810, MIR162 and NK603 confirmed previous analyses (Table 2) indicating that no maize endogenous genes were disrupted by event 1507, MIR162 and NK603, while a predicted maize E3 ubiquitin ligase gene was interrupted by event MON810. The updated bioinformatic analysis on gene interruption provided by the applicant on MON810 event revealed that the sequences flanking the insert correspond to sequences that in the maize reference genome are located 12.5 million bases apart on chromosome 5. As these could suggest a deletion or rearrangement of this region, EFSA requested the applicant to provide additional information to investigate this further and to analyse the safety consequences of the potential genomic deletion or rearrangement. The additional information provided by the applicant, including published proteomic and transcriptomic analyses of MON810, showed no significant differences in the expression of most of the genes located in this region compared to non-GM maize comparators, suggesting that they are present and normally expressed in MON810.

Updated bioinformatic analyses of the amino acid sequence of the newly expressed Cry1F, PAT, Cry1Ab, CP4 EPSPS, CP4 EPSPS L214P, Vip3Aa20 and PMI proteins confirm previous results indicating no significant similarities to toxins and allergens. Updated bioinformatic analyses of the newly created open reading frames (ORFs) within the inserts and spanning the junctions between the insert and the flanking regions for events 1507, MON810, MIR162 and NK603 confirmed previous analyses (Table 2). These analyses indicate that the production of a new peptide showing significant similarities to toxins or allergens for any of the events in maize 1507 × MON810 × MIR162 × NK603 is highly unlikely.

In order to assess the possibility for horizontal gene transfer (HGT) by homologous recombination (HR), the applicant performed a sequence identity analysis with microbial DNA for events 1507, MON810, MIR162 and NK603. The likelihood and potential consequences of plant-to-bacteria gene transfer are described in Section 3.4.4.2.

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

3.3. Systematic literature review

The GMO Panel assessed the applicant's literature searches on maize 1507 × MON810 × MIR162 × NK603, which included a scoping review, according to the guidelines given in EFSA (2010, 2017a, 2019).

A systematic review as referred to in Regulation (EU) No 503/2013 has not been provided in support to the risk assessment of application EFSA-GMO-NL-2015-127. Based on the outcome of the scoping review, the GMO Panel agrees that there is limited value in undertaking a systematic review for maize 1507 × MON810 × MIR162 × NK603 at present.

Although the overall quality of the performed literature searches is acceptable, the GMO Panel considers that future searches on maize 1507 × MON810 × MIR162 × NK603 could be fine-tuned further. The GMO Panel, therefore, recommends the applicant to:

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

None of the relevant publications identified through the literature searches (Appendix C) reported information pointing to safety issues associated with maize 1507 × MON810 × MIR162 × NK603 relevant to the scope of this application.

3.4. Risk assessment of the four-event stack maize 1507 × MON 810 × MIR162 × NK603

3.4.1. Molecular characterisation[8]

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

[8] Dossier: Part II – Section 1.2.2; additional information: 3/5/2016, 26/9/2016 and 10/7/2017.

3.4.1.1. Genetic elements and their biological function

Maize events 1507, MON810, MIR162 and NK603 were combined by conventional crossing to produce the four-event stack 1507 × MON810 × MIR162 × NK603. The structure of the inserts introduced into maize 1507, MON810, MIR162 and NK603 is described in detail in the respective EFSA scientific opinions (Table 2) and no new genetic modifications were involved. Genetic elements in the expression cassettes of the single events are summarised in Table 3.

Intended effects of the inserts in maize 1507 × MON810 × MIR162 × NK603 are summarised in Table 4. Based on the known biological function of the newly expressed proteins (Table 4), the only foreseeable interactions at the biological level are among the Cry proteins or among the Vip3Aa20 and the Cry proteins in susceptible insects, which will be dealt with in Sections 3.4.4.

Table 3: Genetic elements in the expression cassettes of events stacked in maize 1507 × MON 810 × MIR162 × NK603

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
1507	*ubi*ZM1 (*Zea mays*)	–	–	*cry1F* (*Bacillus thurigiensis*)	ORF25PolyA (*Agrobacterium tumefaciens*)
	35S (CaMV)	–	–	*pat* (*Streptomyces viridochromogenes*)	35S (CaMV)
MON810	35S (CaMV) (partial)	I-*Hsp70* (*Z. mays*)	–	*cry1Ab* (*B. thurigiensis*) (partial)	(deleted during integration)
MIR162	ZmUbiInt (*Z. mays*)	–	–	*vip3Aa20* (*B. thuringiensis*)	35S (CaMV)
	ZmUbiInt (*Z. mays*)	–	–	*pmi* (*Escherichia coli*)	*nos* (*A. tumefaciens*)
NK603	*ract1* (*Oryza sativa*)	*ract1* (*O. sativa*)	CTP2 (*Arabidopsis thaliana*)	CP4 *epsps* (*Agrobacterium* sp.)	*nos* (*A. tumefaciens*)
	35S (CaMV)	I-*Hsp70* (*Z. mays*)	CTP2 (*A. thaliana*)	CP4 *epsps l214p* (*Agrobacterium* sp.)	*nos* (*A. tumefaciens*)

UTR: untranslated region; CaMV: cauliflower mosaic virus; CTP: chloroplast transit peptide.
–: When no element was specifically introduced to optimise expression.

Table 4: Characteristics and intended effects of the events stacked in maize 1507 × MON 810 × MIR162 × NK603

Event	Protein	Donor organism and biological function	Intended effects in GM plant
1507	Cry1F	Based on a gene from *B. thuringiensis* subsp. *aizawai*. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (cry) genes (Schnepf et al., 1998)	Event 1507 expresses a truncated version of the Cry1F protein. Cry1F is a protein toxic to certain lepidopteran larvae feeding on maize
	PAT	Based on a gene from *Streptomyces viridochromogenes* Tü494. Phosphinothricin-acetyl-transferase (PAT) enzyme acetylates L-glufosinate-ammonium (Thompson et al., 1987; Wohlleben et al., 1988; Eckes et al., 1989)	Event 1507 expresses the PAT protein which confers tolerance to glufosinate ammonium-based herbicides (Dröge-Laser et al., 1994)
MON 810	Cry1Ab	Based on a gene from *Bacillus thuringiensis* subsp. *kumamotoensis*. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (cry) genes (Schnepf et al., 1998)	Event MON 810 expresses a chimeric, truncated cry1Ab gene. Cry1Ab is a chimeric protein toxic to certain lepidopteran larvae feeding on maize

Event	Protein	Donor organism and biological function	Intended effects in GM plant
MIR162	Vip3Aa20	Based on a gene from *Bacillus thuringiensis* strain AB88 (Estruch et al., 1996). In addition to Cry proteins, *B. thuringiensis* also produces insecticidal proteins during its vegetative growth stage. These are referred to as vegetative insecticidal proteins (Vip) (Fang et al., 2007)	Event MIR162 expresses a modified version of the *B. thuringiensis* vip3Aa1 gene, and encodes Vip3Aa20, a protein toxic to certain lepidopteran larvae feeding on maize
	PMI	Based on a gene from *E. coli*. PMI (phosphomannose isomerase) catalyses the isomerization of mannose-6-phosphate to fructose-6-phosphate and plays a role in the metabolism of mannose (Markovitz et al., 1967)	Event MIR162 expresses PMI, which is used as selectable marker. Mannose normally inhibits root growth, respiration and germination. Transformed cells expressing PMI are able to utilise mannose as a carbon source (Negrotto et al., 2000)
NK603	CP4 EPSPS	Based on a gene from *Agrobacterium* strain CP4 (Barry et al., 2001). 5-Enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) is an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Herrmann, 1995)	Event NK603 expresses the bacterial CP4 EPSPS protein which confers tolerance to glyphosate-containing herbicides as it has lower affinity towards glyphosate than the plant endogenous enzyme
	CP4 EPSPS L214P	Based on a gene from *Agrobacterium* strain CP4 (Barry et al., 2001). 5-Enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) is an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Herrmann, 1995)	Event NK603 expresses a modified version of the bacterial CP4 EPSPS protein which confers tolerance to glyphosate-containing herbicides as it has lower affinity towards glyphosate than the plant endogenous enzyme

3.4.1.2. Integrity of the events in four-event stack

The genetic stability of the inserted DNA over multiple generations in the single maize events 1507, MON810, MIR162 and NK603 was demonstrated previously (see Table 2). Integrity of these events in maize 1507 × MON810 × MIR162 × NK603 was demonstrated by polymerase chain reaction (PCR) and sequence analysis that showed that the sequences of the events (inserts and their flanking regions) in the four-event maize stack are identical to the sequences originally reported for the four single events, thus confirming that the integrity of these events was maintained in the four-event stack maize.

3.4.1.3. Information on the expression of the inserts

Cry1F, PAT, Cry1Ab, Vip3Aa20, PMI and CP4 EPSPS proteins levels were analysed by enzyme-linked immunosorbent assay (ELISA) in material harvested from field trials at four locations in the US in the 2012. Samples analysed included leaf (V6, V9, R1, R4 and R6), root (V9, R1, R4 and R6), whole plant (V9, R1 and R6), pollen (R1), stalk (R1), forage (R4) and grain (R6), both treated and not treated with glyphosate and glufosinate.

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

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

3.4.1.4. Conclusion of the molecular characterisation

The molecular data establish that the events stacked in maize 1507 × MON810 × MIR162 × NK603 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins are similar in the four-event stack and in the single events. Therefore, there is no indication of an interaction that may affect the integrity of the events or the levels of the newly expressed proteins in this stack.

Based on the known biological function of the newly expressed proteins, the only foreseeable interactions at the biological level are among the Cry proteins or among the Vip3Aa20 and the Cry proteins in susceptible insects, which will be dealt with in Section 3.4.4.

3.4.2. Comparative analysis[9]

3.4.2.1. Overview of studies conducted for the comparative analysis

Application EFSA-GMO-NL-2015-127 presents data on agronomic and phenotypic characteristics, as well as on forage and grain/seed composition of maize 1507 × MON810 × MIR162 × NK603 (Table 5).

Table 5: Main comparative analysis studies to characterise GM maize 1507 × MON810 × MIR162 × NK603 provided in the application EFSA GMO NL 2015 127

Study focus	Study details	Comparator	Non-GM reference varieties
Agronomic and phenotypic analysis	Field study, USA and Canada, 2012, twelve sites[(a)]	PHE4N × PHH9H	16[(b)]
	Field study, USA and Canada, 2015, ten sites[(c)]		20[(d)]
Compositional analysis	Field study, USA and Canada, 2012, eight sites[(e)]		14[(f)]

GM: Genetically modified.
(a): The 2012 field trials were located in: Dana, IA; Wyoming, IL; Geneva, MN; York, NE; Germansville, PA; Wall, TX; Delavan, WI; Branchton, Ontario; Kimballton, IA; Stewardson, IL; Rockville, IN and Deerfield, MI.
(b): Non-GM hybrid maize used in the 2012 field trials were 33A46, 3437, 34A15, 34B39, 34F06, 34N61, 35A52, 35Y65, 35K02, 35P12, 36W66, P0423, P0621, P0735, P0751, P0891.
(c): The 2015 field trials were located in Dana, IA; Wyoming, IL; Geneva, MN; York, NE; Germansville, PA; Wall, TX; Delavan, WI; Branchton, Ontario; Kimballton, IA; Stewardson, IL.
(d): Non-GM hybrid maize used in the 2015 field trials were 33A46, 34B39, 34F06, 34H31, 34N61, 34Y02, 35A52, 35F38, 35K02, 35P12, 36B08, 36W66, P0891, P0965, P1028, XL5246, XL5354, XL5475, XL5435, XL6077.
(e): The 2012 field trials were located in Dana, IA; Wyoming, IL; Geneva, MN; York, NE; Germansville, PA; Wall, TX; Delavan, WI; Branchton, Ontario.
(f): Non-GM hybrid maize used in the 2012 field trials were 33A46, 3437, 34A15, 34B39, 34N61, 35A52, 35Y65, 35K02, 35P12, 36W66, P0423, P0621, P0735 and P0751.

Two independent sets of field trials were received: North America (2012) and North America (2015); both sets were considered, although for different purposes. Compositional data were provided only for the field trials in 2012. Agronomic-phenotypic data were measured in both sets, but they were assessed based only on data from the field trials in 2015, as those in 2012 did not include the measurement of yield components (yield and kernel weight) and therefore were considered incomplete. Suitability of materials and representativeness of the receiving environments were assessed for both years. In the following, 'field trials' without specification refers to both 2012 and 2015 datasets, unless noted otherwise.

3.4.2.2. Experimental field trial design and statistical analysis

At each field trial site, the following materials were grown in a randomised complete block design with four replicates: maize 1507× MON810 × MIR162 × NK603 exposed to the intended herbicides glyphosate and glufosinate-ammonium (treated), maize 1507× MON810 × MIR162 × NK603 not exposed to the intended herbicides (not treated), the comparator maize PHE4N×PHH9H and three commercial non-GM maize 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 GM maize 1507 × MON810 × MIR162 × NK603, the application of a difference test (between the GM maize the non-GM

[9] Dossier: Part II – Section 1.3; additional information 26/9/2016; spontaneous additional info 30/5/2016 and 26/9/2016.

comparator) and an equivalence test (between the GM maize and the set of non-GM commercial reference varieties).[10] The results of the equivalence test are categorised into four possible outcomes (I-IV, ranging from equivalence to non-equivalence).[11]

3.4.2.3. Suitability of selected test materials

Selection of the test materials

To obtain the four-event stack maize, the single events 1507, MON810, MIR162 and NK603 were transferred in the genetic background of two different non-GM maize inbred lines, PHE4N and PHH9H. The comparator used in the field trials is the non-GM maize hybrid PHE4N×PHH9H, which has a similar genetic background to that of maize 1507 × MON810 × MIR162 × NK603 (as documented by the pedigree and by the additional information), and is therefore considered to be an acceptable comparator.

GM maize 1507 × MON810 × MIR162 × NK603 and the non-GM comparator, both with a comparative relative maturity (CRM) of 107, are considered appropriate for growing in environments across North America, where the comparative field trials were conducted. The same GM hybrid maize and comparator were used in both 2012 and 2015 field trials.

Commercial non-GM reference varieties with a CRM ranging from 104 to 111 were selected by the applicant and, at each selected site, three reference varieties were tested in the 2012 field trials and four in the 2015 field trials (see Table 5). On the basis of the information provided on relative maturity classes and year of commercialisation, the GMO Panel considers the selected non-GM reference varieties appropriate for the comparative assessment.

Seed production and quality

Seeds of GM maize 1507 × MON810 × MIR162 × NK603 and the conventional comparator used in the 2012 and 2015 field trials were produced from plants harvested and stored under similar conditions, before being sown in the field trial sites. The seed lots were verified for their purity via event specific quantitative PCR analysis. The seed lots of maize 1507 × MON810 × MIR162 × NK603 of its comparator and of two of the non-GM reference varieties[12] were tested for their germination capacity under different temperature conditions.[13] Germination capacity of the maize 1507 × MON810 × MIR162 × NK603 was compared with its comparator. A statistically significant reduction was observed between mean germination rates of 1507 × MON810 × MIR162 × NK603 maize (94.5%) and control maize (98.5%) only under warm growing conditions. The reduction in germination rate was observed only under continuous warm temperature conditions and did not alter the suitability of the materials for the comparative analysis.

Conclusion on suitability

The GMO Panel is of the opinion that the maize 1507 × MON810 × MIR162 × NK603, the comparator and the non-GM maize reference varieties were properly selected and are of adequate quality. Therefore, the test materials are considered acceptable for the comparative analysis.

[10] The purpose of the test of equivalence is to evaluate the estimated mean values for GM maize 1507 × MON810 × MIR162 × NK603 taking into account natural variability as defined by a set of commercial non-GM maize reference varieties with a history of safe use for consumption as food or feed.

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

[12] Non-GM hybrid maize used in the seed germination test: 35A52 and P0751.

[13] Seed germination on F_1 seeds of GM maize and of its conventional counterpart was assessed together with two non-GM reference varieties. Warm temperature condition: 25°C for 7 days. Cold temperature condition: 10°C for 10 days followed by 3 days at 25°C. Diurnal condition: cyclical setting of 10°C for 16 h followed by 25°C for 8 h, repeated daily for 10 days. Non germinated seeds were also assessed with a specific test to verify their viability, and all the non-germinated seeds were confirmed to be also non-viable.

3.4.2.4. Representativeness of the receiving environments

Selection of field trial sites

The selected field trials sites were located in commercial maize-growing regions of United States and Canada. The soil and climatic characteristics of the selected fields were diverse,[14] corresponding to optimal, near-optimal and sub-optimal conditions for maize cultivation (Sys et al., 1993). The GMO Panel considers that the selected sites reflect commercial maize-growing regions in which the test materials are likely to be grown.

Meteorological conditions

Maximum and minimum mean temperatures and sum of precipitations were provided on a monthly and weekly basis for the field trials conducted in 2012 and 2015 respectively. 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.

Management practices

The field trials included plots containing maize 1507 × MON810 × MIR162 × NK603, plots with the comparator and plots with non-GM reference varieties, all managed according to local agricultural practices. In addition, the field trials included plots containing maize 1507 × MON810 × MIR162 × NK603 managed following the same agricultural practices, plus exposed to the intended herbicides. Glyphosate and glufosinate-ammonium containing herbicides were applied as tank mix in two applications at the BBCH 14 and 17 crop growth stages. The GMO Panel considers that the management practices including planting, harvesting and application of plant protection products were acceptable.

Conclusion on representativeness

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

3.4.2.5. Agronomic and phenotypic analysis

Ten[15] agronomic and phenotypic endpoints plus information on abiotic stressors, disease incidence and arthropod damage were collected from ten different trials sites in 2015 (see Table 5). The endpoints lodging and ear count were not analysed with formal statistical methods because of lack of variability in the data.

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

- For maize 1507 × MON810 × MIR162 × NK603 (treated with conventional herbicides), the test of difference identified statistically significant differences with the non-GM comparator for early and final stand count, grain moisture, test weight and yield. All these endpoints fell under equivalence category I or II.
- For maize 1507 × MON810 × MIR162 × NK603 (treated with the intended herbicide), the test of difference identified statistically significant differences with the non-GM comparator for early and final stand count, grain moisture, 100-kernel weight and yield. All these endpoints fell under equivalence category I or II except for early stand count and yield, which fell into equivalence category III.[16]

Early stand count and yield for the four-event stack maize (treated) showed significant differences with respect to the non-GM comparator and fell into equivalence category III. An indication of a possible cause of the observed difference in early stand count come from the observed differences in

[14] Soil types of the full set of field trials were: Loam; Silty/Clay/Loam; Silt/Loam; Sandy/Clay/Loam; Clay/Loam; Sandy Loam and Clay. Soil organic carbon and pH of the 2015 field trials ranged respectively from 0.9% to 3.1% and from 5.7 to 7.9. Average temperatures and sum of precipitations during the usual crop growing season ranged respectively from 15.0°C to 24.7°C and from 386 mm to 826mm for 2012 field trials and from 12.5°C to 23.5°C and from 409 mm to 905mm for 2015 field trials.

[15] Early stand count, days to flowering, plant height, days to maturity, lodging, final stand count, ear count, grain moisture, seed weight and yield.

[16] The estimated mean values for early stand count (plants/plot) were: 92 (treated GM); 98.4 (comparator), 98.2 (reference varieties); equivalence limits: 92.4-102. The estimated mean values for yield (t/ha) were: 9.3 (treated GM), 10.8 (comparator), 10.7 (reference varieties); equivalence limits: 9.5-11.9.

seed germination (see section 3.4.2.3). Whether the differences can lead to an environmental adverse effect is considered in Section 3.4.4.

3.4.2.6. Compositional analysis

Maize forage and grains harvested from the field trials in the USA and Canada in 2012 were analysed for 84 different constituents (nine in forage and 75 in grains), including the key constituents recommended by the Organisation for Economic Co-operation and Development (OECD) (OECD, 2002). Thirteen grain constituents having more than 50% of the observations below the limit of quantification were excluded from the statistical analysis.[17]

The test of difference and the test of equivalence could be applied to the remaining 71 constituents (nine in forage[18] and 62 in grains[19]), with the following results (Table 6):

- For maize 1507 × MON810 × MIR162 × NK603 (not treated), the test of difference identified statistically significant differences from the non-GM comparator for 40 constituents (two in forage and 38 in grains). The test of equivalence between maize 1507 × MON810 × MIR162 × NK603 and the non-GM maize reference varieties indicated that all 40 constituents fell under equivalence category I or II.
- For maize 1507 × MON810 × MIR162 × NK603 (treated), statistically significant differences were also identified for 40 constituents (3 in forage and 37 in grains). All 40 constituents fell under equivalence category I or II.

Table 6: Outcome of the comparative compositional analysis in grains and forage for maize 1507 × MON810 × MIR162 × NK603. 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	31	40[(d)]	31	40[(d)]
	Category III/IV	–	–	–	–
	Total endpoints	71			71

(a): Comparison between maize 1507 × MON810 × MIR162 × NK603 and the non-GM comparator.
(b): Four different outcomes: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence).
(c): Not treated/treated with the intended herbicides glyphosate and glufosinate ammonium (Section 3.4.2.2).
(d): Endpoints with significant differences between maize 1507 × MON810 × MIR162 × NK603 and the non-GM comparator falling under equivalence category I-II (treated and not treated). In grain, both treated and not treated: alanine, aspartic acid, glutamic acid, isoleucine, leucine, phenylalanine, serine, threonine, valine, phytic acid, palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linolenic acid (C18:3), eicosenoic acid (C20:1), copper, iron, phosphorus, potassium, ADF, ash, carbohydrates, crude fat, crude fibre, crude protein, moisture, NDF, ferulic acid, p-coumaric acid, pyridoxine, γ-tocopherol; only treated: glycine, histidine, proline, tyrosine, β-carotene; only not treated: lysine, trypsin inhibitor, lignoceric acid (C24:0), magnesium, total tocopherols, thiamine. In forage, both treated and not treated: neutral detergent fibre (NDF); only treated: crude fat, crude fibre; only not treated: calcium.

[17] These were: caprylic acid (C8:0), lauric acid (C12:0), myristic acid (C14:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), heptadecenoic acid (C17:1), heptadecadienoic acid (C17:2), (9,15) isomer of linoleic acid (C18:2), nonadecanoic acid (C19:0), eicosadienoic acid (C20:2), heneicosanoic acid (C21:0), behenic acid (C22:0), tricosanoic acid (C23:0).

[18] Crude protein, crude fat, crude fibre, acid detergent fibre (ADF), neutral detergent fibre (NDF), ash, carbohydrates, calcium, phosphorus.

[19] Moisture, crude protein, crude fat, crude fibre, acid detergent fibre (ADF), neutral detergent fibre (NDF), ash, carbohydrates, alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, calcium, manganese, phosphorus, iron, magnesium, copper, potassium, sodium, zinc, 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), palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), heptadecenoic acid (C17:1), heptadecadienoic acid (C17:2), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), (9,15) isomer of linoleic acid (C18:2), linolenic acid (C18:3), γ-linolenic acid (C18:3), nonadecanoic acid (C19:0), arachidic acid (C20:0), eicosenoic acid (C20:1), eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3), arachidonic acid (C20:4), heneicosanoic acid (C21:0), behenic acid (C22:0), erucic acid (C22:1), tricosanoic acid (C23:0), lignoceric acid (C24:0), β-carotene, thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, folic acid, α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol, *p*-coumaric acid, inositol, ferulic acid, furfural, phytic acid, raffinose, trypsin inhibitor.

The GMO Panel assessed all the significant differences between maize 1507 × MON810 × MIR162 × NK603 and its non-GM comparator, taking into account the potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties. No endpoints showing significant differences between the four-event stack maize and the non-GM comparator and falling under category III/IV were identified.

3.4.2.7. Conclusion on comparative analysis

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

- The differences identified in the agronomic and phenotypic characteristics tested between the four-event stack maize and the non-GM comparator do not need further assessment for environmental safety, with the exception of the changes noted in regard to early stand count and yield, which are considered in Section 3.4.4.
- The differences identified in forage and grain composition between the four-event stack maize and the non-GM comparator do not need further assessment in regard to food and feed safety.

3.4.3. Food/Feed safety assessment

3.4.3.1. Effects of processing

Processed products

Maize 1507 × MON810 × MIR162 × NK603 will undergo existing production processes used for conventional maize. No novel production process is envisaged. Based on the outcome of the comparative assessment, processing of the four-event stack maize 1507 × MON810 × MIR162 × NK603 into food and feed products is not expected to result in products being different from those of conventional non-GM maize varieties.

3.4.3.2. Influence of Temperature and pH on newly expressed proteins

Effects of temperature and pH on the newly expressed proteins in this four-event stack maize have been previously evaluated by the GMO Panel (Table 2). No additional studies were provided in the context of this application.

3.4.3.3. Toxicology

Testing of newly expressed proteins

Six proteins (Cry1F, Cry1Ab, Vip3Aa20, PAT, PMI and CP4 EPSPS, including the variant CP4 EPSPS L214P) are newly expressed in the four-event stack maize 1507 × MON810 × MIR162 × NK603 (Section 3.4.1). The GMO Panel has previously assessed these proteins in the context of the single events (Table 2), and no safety concerns were identified for humans and animals. The GMO Panel is not aware of any new information that would change this conclusion.

The potential for a functional interaction among the proteins newly expressed in maize 1507 × MON810 × MIR162 × NK603 has been assessed with regard to human and animal health. The three enzymatic proteins (CP4 EPSPS, PAT and PMI) catalyse distinct biochemical reactions, acting on unrelated substrates and are not expected to interact. The CP4 EPSPS proteins act on the shikimic acid pathway for the biosynthesis of aromatic amino acids in plants, showing high substrate specificity. PAT acts on the herbicide glufosinate and PMI (used as selectable marker) is involved in carbohydrate metabolism in plant, allowing maize cells to use mannose as a sole carbon source.

The insecticidal proteins Cry1F and Cry1Ab are delta-endotoxins acting through cellular receptors found in target insect species. It is reported that the gastrointestinal tract of mammals, including humans, lacks receptors with high specific affinity to Cry proteins (Hammond et al., 2013; Koch et al., 2015). The Vip3Aa20 protein is a protein secreted by *B. thuringiensis* during its vegetative phase acting in target insects via a mechanism similar to that of Cry proteins (Chakroun et al., 2016; Bel et al., 2017).

On the basis of the known biological function of the individual newly expressed proteins (Table 4), there is currently no expectation for possible interactions relevant to the food and feed safety of the four-event stack maize 1507 × MON810 × MIR162 × NK603.

In vitro protein degradation studies on Cry1F, Cry1Ab, Vip3Aa20, PAT, PMI and CP4 EPSPS (including its variant CP4 EPSPS L214P) proteins have been previously evaluated by the GMO Panel (Table 2). No new information has been provided in the context of this application.

The GMO Panel concludes that there are no safety concerns to human and animal health related to the newly expressed proteins Cry1F, Cry1Ab, Vip3Aa20, PAT, PMI, CP4 EPSPS and its variant CP4 EPSPS L214P in the four-event stack maize 1507 × MON810 × MIR162 × NK603.

Testing of new constituent other than proteins

No new constituents other *than newly expressed proteins have been identified in in grain and forage from the four-event stack maize 1507 × MON810 × MIR162 × NK603. Therefore, no further food/feed safety assessment of components other than the newly expressed proteins is required.*

Information on altered levels of food and feed constituent

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

Testing of the whole genetically modified food and feed

Based on the outcome of the molecular characterisation, comparative analysis and toxicological assessment, no indication of findings relevant to food/feed safety related to the stability and expression of the inserts or to interaction between the transformation events, and no modifications of toxicological concern in the composition of maize 1507 × MON810 × MIR162 × NK603 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 four-stack are not necessary (EFSA GMO Panel, 2011a).

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

90-Day studies on maize MIR162 and NK603

The GMO Panel had previously concluded that these studies are in line with Regulation (EU) No 503/2013 and do not show adverse effects related to diets incorporating the single-event maize MIR162 and NK603 (EFSA GMO Panel, 2019c).

90-Day studies on maize MON810

A 90-day study on maize MON810 had been previously assessed by the GMO Panel in the context of the single-event renewal application dossier (EFSA, 2009a). Upon EFSA's request to fulfil the requirements of Regulation (EU) No 503/2013, the applicant provided details of the histopathological findings of a number of organs and tissues, not originally examined according to OECD test guideline 408 (OECD, 1998).[20] Sporadic histopathological findings were seen in the additional tissues and organs examined both in male and female rats, compatible with the spontaneous background pathology of rats of this strain and age. A spontaneous mammary adenocarcinoma reported in a single female (F2019) fed diet containing grains (33%) from maize MON810 was concluded to be an incidental occurrence. The GMO Panel concludes that this study is in line with the legal requirements and confirms that there are no indications of adverse effects related to the 90-day administration to rats of diets including grains from maize MON810. The incorporation rate of maize selected in the study with maize MON810 is up to 33%, in line with commercially available rodent diets. It has been recently reported that a diet incorporating 50% maize may be tolerated without inducing nutritional imbalances in rats after 90-day administration (Steinberg et al., 2019), but the GMO Panel considers that further scientific confirmation is needed before this 50% maize incorporation rate is applicable in future studies.

90-Day study on maize 1507[21]

The GMO Panel had previously assessed a 90-day study on maize 1507 in the context of the single-event application (EFSA, 2005a). Kernels used in that study were obtained from 1507 maize plants that had not been treated with the intended herbicide (glufosinate-based herbicide). Upon EFSA's request to fulfil the requirements of Regulation (EU) No 503/2013, the applicant provided a new 90-day study on 1507 maize. Pair-housed Crl:CD(SD) rats (16/sex per group; 2 rats/cage) were allocated to six groups using a randomised complete block design with 5 replications/sex. Groups were fed diets containing 50% by weight grains either from maize 1507 plants treated with the intended herbicide

[20] Additional information 31/10/2018.
[21] Additional information 24/2/2020 and 13/7/2020.

(test material, high dose), from the conventional counterpart (control material), or one of three non-transgenic commercial reference maize hybrids.[22] An additional group was fed diets containing 33% by weight grains from maize 1507 treated with the intended herbicide (test material, low dose) and 17% by weight maize grain from the conventional counterpart. The study was adapted from OECD test guideline 408 (OECD, 2018), aligned with EFSA Scientific Committee guidance (EFSA Scientific Committee, 2011) and complied with the principles of good laboratory practice (GLP) with some deviations not impacting the study results and interpretation (i.e. test item stability, homogeneity and concentration), which are detailed below. Event-specific PCR analysis confirmed the presence of the event 1507 in both the GM maize grains and diets and excluded the presence of the event in the respective controls. ELISA analyses also confirmed the presence of event DAS-1507 (i.e. Cry1F concentration) in the GM maize grains and GM diets. Both GM and control maize grains and diets were analysed for nutrients, antinutrients and potential contaminants (e.g. selected heavy metals, mycotoxins and pesticides). Balanced diets were formulated based on the specifications for PMI Certified Rodent LabDiet® 5002. The stability of the test and control materials was not verified; however, in accordance to product expiration 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. The applicant provided information on concentration of Cry1F protein in the formulated test diets, further supporting the homogeneity of the formulations. Feed and water were provided *ad libitum*. In-life procedures and observations and terminal procedures were conducted in accordance to OECD test guideline 408 (OECD, 2018).

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

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

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

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

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

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

3.4.3.4. Allergenicity

For allergenicity assessment, a weight-of-evidence approach was followed, taking into account all of the information obtained on the newly expressed proteins, as no single piece of information or

[22] P0760, P0589 and XL5840.

experimental method yields sufficient evidence to predict allergenicity and adjuvanticity (Codex Alimentarius, 2009; EFSA GMO Panel, 2011a; Commission Regulation (EU) No 503/2013).

Assessment of allergenicity of the newly expressed proteins

For allergenicity, the GMO Panel has previously evaluated the safety of the Cry1F, Cry1Ab, Vip3Aa20, PAT, PMI and CP4 EPSPS (including the variant CP4 EPSPS L214P) 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 newly expressed proteins in this four-event stack maize that might change the previous conclusions of the EFSA GMO Panel has become available. Based on 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 potential interaction in this four-event stack maize.

Furthermore, the GMO Panel has previously evaluated the safety of the newly expressed proteins, and no concerns on adjuvanticity in the context of the applications assessed were identified (Table 2). More recently, this aspect has been discussed in detail by EFSA (EFSA, 2018b; Parenti et al., 2019). To date there is no evidence for adjuvanticity in the GMOs assessed by the Panel. This four-event stack maize has comparable 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 four-event stack maize might be adjuvants able to enhance an allergic reaction.

Assessment of allergenicity of GM plant products

The GMO Panel regularly reviews the available publications on food allergy to maize. However, maize is not considered a common allergenic food[23] (OECD, 2002). Therefore, the GMO Panel does not request experimental data to analyse the allergen repertoire of GM maize.

In the context of this application and considering the data from the molecular characterisation, the compositional analysis and the assessment of the newly expressed proteins (Sections 3.4.1, 3.4.2 and 3.4.3), the GMO Panel identified no indications of a potentially increased allergenicity of food and feed derived from this four-event stack maize with respect to that derived from its non-GM comparator.

3.4.3.5. Dietary exposure assessment to new constituents

In line with Regulation (EU) No 503/2013 the applicant provided dietary exposure estimates to Cry1F, PAT, Cry1Ab, Vip3Aa20, PMI and CP4 EPSPS proteins newly expressed in 1507 × MON810 × MIR162 × NK603 maize. Dietary exposure was estimated based on protein expression levels reported in this application for the four-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 1507 × MON810 × MIR162 × NK603 maize grains, forage and pollen were derived from replicated field trials (four replicates from four locations) in the 2012 US growing season (see section 3.4.2). When for a particular newly expressed protein all the data were below the limit of quantification (LOQ), this LOQ was used as expression value to estimate dietary exposure. Table 7 describes the protein expression levels used to estimate both human and animal dietary exposure.

[23] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

Table 7: Mean values (n = 16, μg/g dry weight and μg/g fresh weight) for newly expressed proteins in grains, forage and pollen from 1507 × MON810 × MIR162 × NK603 maize treated with the intended herbicides[(a)]

Protein	Tissue/developmental stage		
	Grains/R6 (μg/g dry weight)	Pollen/R1 (μg/g fresh weight)[(b)]	Forage/R4 (μg/g dry weight)
Cry1F	3.2	29.1	14
PAT	< LOQ[(c)]	< LOQ[(c)]	1.1
Cry1Ab	0.18	< LOQ[(c)]	7.1
Vip3Aa20	49	132	250
PMI	1.6	5.2	5.3
CP4 EPSPS	12	489	140

LOQ: limit of quantification.
(a): Intended herbicide: glufosinate and glyphosate.
(b): Concentrations values in pollen were adjusted to 6% moisture content before using them to estimate dietary exposure to the different newly expressed protein via the consumption of pollen supplements.
(c): All samples were below the limit of quantification: for PAT protein in grain (LOQ = 0.069 μg/g dry weight), for PAT protein in pollen (LOQ = 0.26 μg/g fresh weight), for Cry1Ab protein in pollen (LOQ = 0.16 μg/g fresh weight).

Human dietary exposure[24]

As per request of the GMO Panel, chronic and acute dietary exposure to the newly expressed proteins in 1507 × MON810 × MIR162 × NK603 maize grains were provided. The applicant followed the methodology described by EFSA to estimate dietary exposure in high consumers using summary statistics (EFSA, 2015).

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 1507 × MON810 × MIR162 × NK603 maize grains, a conservative scenario with 100% replacement of conventional maize by the GM maize was considered. Consumption figures for all relevant commodities (e.g. corn flakes, sweet corn, popcorn, etc.) were retrieved from the EFSA Comprehensive European Food Consumption Database (EFSA consumption database).[25] Corn oil was excluded from the assessment since no proteins are expected to be present in the oil.

Mean protein expression values on fresh weight basis are considered as the most adequate to estimate human dietary exposure (EFSA, 2019). However, dietary exposure was provided using expression values on dry weight basis; this results in more conservative exposure estimations which is considered acceptable. Since Cry1F, PAT, Cry1Ab, Vip3Aa20, PMI and CP4 EPSPS proteins were not analysed in processed foods, the concentration of the newly expressed proteins in these commodities was estimated using the ratio between the total protein content in processed foods and in maize grains.[26] This is a conservative approach as neither recipes nor the effect of processing is considered on the final concentration of newly expressed proteins, except for corn oil which is eventually excluded from the exposure estimations.

The highest acute dietary exposure was estimated in the age class 'Toddlers' with exposure estimates of 48.1 μg/kg body weight (bw) per day, 1.0 μg/kg bw per day, 2.7 μg/kg bw per day, 737 μg/kg bw per day, 24.1 μg/kg bw per day and 180 μg/kg bw per day for Cry1F, PAT, Cry1Ab, Vip3Aa20, PMI and CP4 EPSPS, respectively. The main average contributor to the exposure in the dietary survey with the highest estimates was corn chips.

The highest chronic dietary exposure was estimated in the age class 'Infants' with exposure estimates of 10.8 μg/kg bw per day, 0.2 μg/kg bw per day, 0.6 μg/kg bw per day, 165 μg/kg bw per day, 5.4 μg/kg bw per day, and 40.5 μg/kg bw per day for Cry1F, PAT, Cry1Ab, Vip3Aa20, PMI and CP4 EPSPS, respectively. The main average contributor to the exposure in the dietary survey with the highest estimates was corn flour.

[24] Additional information: 7/5/2018.
[25] https://www.efsa.europa.eu/en/applications/gmo/tools. Data accessed April 2018.
[26] The protein content of maize grain and maize foods was obtained from the EuroFIR Food Composition Databases.

An ad hoc dietary exposure scenario was carried out for consumers of pollen supplements under the assumption that these supplements might be made of pollen from 1507 × MON810 × MIR162 × NK603 maize. Consumption data on pollen supplements are available for few consumers across nine different European countries[27]; the low number of consumers available adds uncertainty to the exposure estimations and prevents to estimate exposure for high consumers of pollen supplements. In average consumers of pollen supplements, the highest acute dietary exposure would range from 0.12 μg/kg bw per day for Cry1Ab to 362 μg/kg bw per day for CP4 EPSPS, in the elderly population. Similarly, the highest chronic dietary exposure in average consumers would range from 0.08 μg/kg bw per day for Cry1Ab to 242 μg/kg bw per day for CP4 EPSPS, also in the elderly population.

Animal dietary exposure

Dietary exposure to Cry1F, PAT, Cry1Ab, Vip3Aa20, PMI and CP4 EPSPS proteins in maize 1507 × MON810 × MIR162 × NK603 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 and forage). A conservative scenario with 100% replacement of conventional maize products by the four-event stack maize products was considered.

Mean levels (dry weight) of the newly expressed proteins in grains and forage from the four-event stack maize treated with the intended herbicide used for animal dietary exposure are listed in Table 7.

The applicant estimated dietary exposure to Cry1F, PAT, Cry1Ab, Vip3Aa20, PMI and CP4 EPSPS proteins in livestock (i.e. poultry, swine, cattle and sheep), based on estimates for body weights, daily feed intakes and inclusion rates (percentage) of maize grains and forage in diets/rations (OECD, 2009).

Estimated dietary exposure in livestock animals was calculated based on the consumption of maize grain and forage alone or in combination, as reported in Appendix E.

3.4.3.6. Nutritional assessment of endogenous constituents

The intended traits of 1507 × MON810 × MIR162 × NK603 maize are herbicide- and insect resistance, with no intention to alter nutritional parameters. Comparison of the composition of the four-event stack maize, with the non-GM comparator and non-GM reference varieties did not identify differences that would require further safety assessment. From these data, the GMO Panel concludes that 1507 × MON810 × MIR162 × NK603 maize is nutritionally equivalent to the non-GM comparator and the non-GM reference varieties used.

3.4.3.7. Conclusion of the food and feed safety assessment

The newly expressed proteins Cry1F, PAT, Cry1Ab, Vip3Aa20, PMI and CP4 EPSPS proteins in 1507 × MON810 × MIR162 × NK603 maize do not raise safety concerns for human and animal health. Interactions among the newly expressed proteins Cry1F, PAT, Cry1Ab, Vip3Aa20, PMI and CP4 EPSPS proteins raising food and feed safety concerns (in terms of toxicology, allergenicity and adjuvanticity) are not expected. There is no evidence that the genetic modification might change the overall allergenicity of the four-event stack maize. Based on the outcome of the animal and human nutritional assessments, the consumption of 1507 × MON810 × MIR162 × NK603 maize does not represent any nutritional concern, in the context of the scope of this application.

3.4.4. Environmental risk assessment

Considering the scope of application EFSA-GMO-NL-2015-127, which excludes cultivation, the environmental risk assessment (ERA) of maize 1507 × MON810 × MIR162 × NK603 mainly takes into account: 1) the exposure of microorganisms to recombinant DNA in the gastrointestinal tract of animals fed GM material and of microorganisms present in environments exposed to faecal material of these animals (manure and faeces); and 2) the accidental release into the environment of viable four-events stack maize grains during transportation and/or processing (EFSA GMO Panel, 2010a).

3.4.4.1. Persistence and invasiveness of the GM plant

Maize is highly domesticated, not winter hardy in colder regions of Europe, and generally unable to survive in the environment without appropriate management. Occasional feral GM maize plants may occur outside cultivation areas in the EU (e.g. Pascher, 2016), but survival is limited mainly by a combination of low competitiveness, absence of a dormancy phase and susceptibility to plant

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

pathogens, herbivores and cold climate conditions (OECD, 2003). Field observations indicate that maize grains may survive and overwinter in some EU regions, resulting in volunteers in subsequent crops (e.g. Gruber et al., 2008; Palaudelmàs et al., 2009; Pascher, 2016). However, maize volunteers have been shown to grow weakly and flower asynchronously with the maize crop (Palaudelmàs et al., 2009). Thus, the establishment and survival of feral and volunteer maize in the EU is currently limited and transient.

It is unlikely that the intended traits of event 1507 × MON810 × MIR162 × NK603 will provide a selective advantage to maize plants, except when they are exposed to glyphosate- and/or glufosinate-containing herbicides or infested by insect pests that are susceptible to the Cry1F, Cry1Ab and/or Vip3Aa20 proteins.

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

In conclusion, the GMO Panel considers it unlikely that maize 1507 × MON810 × MIR162 × NK603 will differ from conventional maize hybrid varieties in its ability to survive until subsequent seasons, or to establish occasional feral plants under European environmental conditions in case of accidental release into the environment of viable maize 1507 × MON810 × MIR162 × NK603 grains.

3.4.4.2. Potential for gene transfer

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

Plant-to-microorganism gene transfer

The probability and potential adverse effects of HGT of the recombinant DNA have been assessed in previous GMO Panel Scientific Opinions for the single events (see Table 2). This assessment included consideration of homology-based recombination processes, as well as non-homologous end joining and microhomology-mediated end joining. Possible fitness advantages that the bacteria in the receiving environments would gain from acquiring recombinant DNA were considered. No concern as a result of an unlikely, but theoretically possible, HGT of the recombinant genes to bacteria in the gut of domesticated animals and humans fed GM material or other receiving environments was identified.

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

The updated bioinformatics analyses of events 1507, MIR162 and NK603 do not reveal any new DNA sequence that could provide sufficient length and identity which could facilitate HGT by double homologous recombination, confirming the conclusions of previous Scientific Opinions (EFSA GMO Panel, 2019b,d,e).

The updated bioinformatic analysis of MON810 confirmed the absence of sequences in the recombinant DNA which would provide sufficient sequence identity to facilitate HGT to bacteria.

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

Plant-to-plant gene transfer

The potential for occasional feral maize 1507 × MON810 × MIR162 × NK603 plants originating from grain import spills to transfer recombinant DNA to sexually compatible plants and the environmental consequences of this transfer were considered.

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

Maize is an annual predominantly cross-pollinating crop. Cross-fertilisation occurs mainly by wind (OECD, 2003). Vertical gene transfer from maize is limited to *Zea* species. Wild relatives of maize outside cultivation are not known/reported in Europe (Eastham and Sweet, 2002; EFSA, 2016; OECD,

2003; 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; Le Corre et al., 2020; Trtikova et al., 2017).

The potential of spilled maize grains to establish, grow and produce pollen is extremely low and transient (see Section 3.4.4.1). Therefore, the likelihood/frequency of cross-pollination between occasional feral GM maize plants resulting from grain spillage, and weedy or cultivated *Zea* plants is considered extremely low (EFSA, 2016). Even if cross-pollination would occur, the GMO Panel is of the opinion that environmental effects as a consequence of the spread of genes from occasional feral GM maize plants in Europe will not differ from that of conventional maize varieties 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 the application EFSA-GMO-NL-2015-127 into account (no cultivation), potential interactions of occasional feral four-event stack maize plants arising from grain import spills with the target organisms are not considered a relevant issue by the GMO Panel.

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

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

3.4.4.5. Interactions with abiotic environment and biogeochemical cycles

Given that environmental exposure to spilled grains or occasional feral four-event stack maize plants arising from grain import spills is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions with the abiotic environment and biogeochemical cycles are not considered by the GMO Panel to raise any environmental safety concern.

3.4.4.6. Conclusion of the environmental risk assessment

The GMO Panel concludes that it is unlikely that the four-event stack maize would differ from conventional maize varieties in its ability to persist under EU environmental conditions. Considering the scope of the application EFSA-GMO-NL-2015-127, interactions of occasional feral four-event stack maize plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from the four-event stack maize to bacteria does not indicate a safety concern. Therefore, considering the combined traits and their interactions, the outcome of the agronomic and phenotypic analysis, and the routes and levels of exposure, the GMO Panel concludes that the four-event stack maize would not raise safety concerns in the event of accidental release of viable GM maize grains into the environment.

3.4.5. Conclusion on the four-event stack maize 1507 × MON810 × MIR162 × NK603

No new data on the four single maize events 1507, MON810, MIR162 and NK603 that would lead to a modification of the original conclusions on their safety were identified.

The combination of maize events 1507, MON810, MIR162 and NK603 in the four-event stack maize did not give rise to issues concerning the molecular, agronomic, phenotypic or compositional characteristics of the four-event stack maize that would be of concern for food and feed safety and nutrition.

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

No indications of interactions among the events based on the biological functions of the newly expressed proteins that would raise a safety issue were identified in maize 1507 × MON810 × MIR162 × NK603. Comparison of the levels of the newly expressed proteins between the four-event stack maize and those of the single maize events did not reveal an interaction at protein expression level.

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

No scientific information that could change the conclusions on this four-event stack maize was retrieved through systematic literature searches covering the 10 years before submission of the application and the period since the time of validity of the application. The GMO Panel concludes that maize 1507 × MON810 × MIR162 × NK603, as described in this application, is nutritionally equivalent to and as safe as the comparator and the non-GM reference varieties tested.

3.5. Risk assessment of the subcombinations[24]

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

3.5.1. Subcombinations previously assessed

The GMO Panel has previously assessed six subcombinations and no safety concerns were identified: the two-event maize stacks MON810 × 1507, MON810 × NK603, 1507 × NK603, 1507 × MIR162 and NK603 × MIR162; the three-event stack maize MON810 × 1507 × NK603 and all its subcombinations (see Table 2). Literature searches covering the 10 years before submission of the application and the period since the time of validity of the application revealed no new scientific information relevant to the risk assessment of these maize stacks.[28] Consequently, the GMO Panel considers that its previous conclusions on these subcombinations remain valid.

3.5.2. Subcombinations not previously assessed

Four of the 10 subcombinations included in the scope of this application have not been previously assessed by the GMO Panel (Table 8). In this case, following the strategy defined by the GMO Panel,[29] the risk assessment takes as its starting point the assessment of the single maize events, and uses the data generated for the four-event stack as well as all the additional data available on subcombinations previously assessed by the GMO Panel (Table 2) and the additional studies provided by the applicant (Appendix A).

Table 8: Maize stacks not previously assessed and covered by the scope of application EFSA-GMO-NL-2015-127

Degree of stacking	Events
Three-event stack	1507 × NK603 × MIR162
	MON 810 × NK603 × MIR162
	MON 810 × 1507 × MIR162
Two-event stack	MON 810 × MIR162

3.5.2.1. Stability of the events

The genetic stability of the inserted DNA over multiple generations in the four single maize events was demonstrated previously (see Table 2). Integrity of the events was demonstrated in the four-event stack maize 1507 × MON810 × MIR162 × NK603 (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 four events by conventional crossing could result in significant changes in expression levels of the newly expressed proteins, as this could indicate an unexpected interaction 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

[28] Dossier: Part II – Section 7; additional information: 17/11/2017, 8/1/2018 and 13/7/2020.
[29] 115th GMO Panel meeting (Annex 1 of the minutes: http://www.efsa.europa.eu/sites/default/files/event/170517-m.pdf).

expressed proteins in the four subcombinations compared with those in the single maize events. This assumption was confirmed by comparing the levels of the newly expressed proteins of each single maize event with those of the four-event stack maize. The levels were similar in the four-event stack maize and in the single events (Section 3.4.1.3 and Appendix B). Therefore, there was no indication of an interaction at protein expression level. In addition, expression data from the two-event stack maize NK603 × MON810 (EFSA, 2005c,d; EFSA GMO Panel, 2018a) and 1507 × NK603 (EFSA, 2006; EFSA GMO Panel, 2018b) were similar to those observed in each of the single maize events. This supports the conclusion that interactions affecting the expression levels of the newly expressed proteins are not expected in the four subcombinations not previously assessed and included in the scope of application EFSA-GMO-NL-2015-127.

3.5.2.3. Potential functional interactions among the events

The GMO Panel assessed the potential for interactions among maize events in the 4 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 four single events, the previously assessed subcombinations (Table 2) and the four-event stack maize. It is concluded that none of these events would raise safety concerns when combined in any of these maize subcombinations. The GMO Panel considers that no further data are needed to complete the assessment of subcombinations from the four-event stack maize.

3.5.3. Conclusion

Since no new safety concerns were identified for the previously assessed subcombinations, the GMO Panel considers that its previous conclusions on these maize subcombinations remain valid. For the remaining 4 subcombinations included in the scope of application EFSA-GMO-NL-2015-127, 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 four-event stack maize.

3.6. Post-market monitoring

3.6.1. Post-market monitoring of GM food/feed

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

3.6.2. Post-market environmental monitoring

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

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

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

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

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

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

3.6.3. Conclusion on post-market monitoring

No PMM of food and feed is necessary. The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of maize 1507 × MON810 × MIR162 × NK603.

4. Overall conclusions

The GMO Panel was asked to carry out a scientific assessment of maize 1507 × MON810 × MIR162 × NK603 and subcombinations for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003.

No new information on the four single maize events 1507, MON810, MIR162 and NK603 that would lead to a modification of the original conclusions on their safety were identified.

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

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

Since no new data on the six subcombinations previously assessed that would lead to a modification of the original conclusions on their safety were identified, the GMO Panel considers that its previous conclusions on these maize stacks remain valid. For the remaining four subcombinations included in the scope of application EFSA-GMO-NL-2015-127, no information has been provided. The GMO Panel assessed possible interactions among the events in the four subcombinations, and concludes that these combinations of events 1507, MON810, MIR162 and NK603 would not raise safety concerns. These subcombinations are therefore expected to be as safe as and nutritionally equivalent to the maize single events, the previously assessed subcombinations and the four-event stack maize.

Based on the relevant publications identified through the literature searches, the GMO Panel did not identify any safety issues pertaining to the intended uses of maize 1507 × MON810 × MIR162 × NK603 and its subcombinations. In the context of annual PMEM reports, the applicant could further fine-tune future literature searches according to the GMO Panel recommendations.

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

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

In conclusion, the GMO Panel considers that maize 1507 × MON810 × MIR162 × NK603 and its subcombinations, as described in this application, are as safe as the non-GM comparator and the

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

5. Documentation as provided to EFSA

1) Letter from the Competent Authority of the Netherlands received on 18 December 2015 concerning a request for authorization of the placing on the market of maize 1507 × MON 810 × MIR162 × NK603 submitted in accordance with Regulation (EC) No 1829/2003 by Pioneer Overseas Corporation.
2) Application EFSA-GMO-NL-2015-127 validated by EFSA, 09 February 2016.
3) Request for supplementary information to the applicant, 12 February 2016.
4) Request for supplementary information to the applicant, 08 March 2016.
5) Receipt of supplementary information from the applicant, 03 May 2016.
6) Receipt of spontaneous information from the applicant, 30 May 2016.
7) Receipt of spontaneous information from the applicant, 06 June 2016.
8) Request for supplementary information to the applicant, 06 July 2016.
9) Receipt of supplementary information from the applicant, 26 September 2016.
10) Request for supplementary information to the applicant, 15 November 2016.
11) Request for supplementary information to the applicant, 07 March 2017.
12) Request for supplementary information to the applicant, 08 March 2017.
13) Request for supplementary information to the applicant, 18 May 2017.
14) Receipt of supplementary information from the applicant, 09 June 2017.
15) Receipt of supplementary information from the applicant, 10 July 2017.
16) Request for supplementary information to the applicant, 05 September 2017.
17) Request for supplementary information to the applicant, 10 October 2017.
18) Receipt of supplementary information from the applicant, 17 November 2017.
19) Request for supplementary information to the applicant, 13 November 2017.
20) Receipt of spontaneous information from the applicant, 17 November 2017.
21) Receipt of spontaneous information from the applicant, 17 November 2017.
22) Request for supplementary information to the applicant, 13 December 2017.
23) Receipt of supplementary information from the applicant, 08 January 2018.
24) Receipt of spontaneous information from the applicant, 10 January 2018.
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27) Request for supplementary information to the applicant, 22 March 2018.
28) Receipt of supplementary information from the applicant, 07 May 2018.
29) Receipt of supplementary information from the applicant, 06 September 2018.
30) Request for supplementary information to the applicant, 06 September 2018.
31) Request for supplementary information to the applicant, 14 September 2018.
32) Receipt of supplementary information from the applicant, 05 October 2018.
33) Receipt of spontaneous information from the applicant, 05 October 2018.
34) Receipt of supplementary information from the applicant, 30 October 2018.
35) Receipt of spontaneous information from the applicant, 17 December 2018.
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Abbreviations

ADF	acid detergent fibre
bw	body weight
CaMV	cauliflower mosaic virus
CRM	comparative relative maturity
CTP	chloroplast transit peptide
ELISA	enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphat synthase
ERA	environmental risk assessment
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 protein
LOQ	limit of quantification
NDF	neutral detergent fibre
Nos	nopaline synthase
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PAT	phosphinothricin-acetyl-transferase
PCR	polymerase chain reaction
PMEM	post-market environmental monitoring
PMI	phosphomannose isomerase
ract	rice actin
TSH	thyroid hormones
US	United States
UTR	untranslated region

Appendix A – List of additional studies

List of additional studies performed by or on behalf of the applicant with regard to the evaluation of the safety of maize 1507 × MON810 × MIR162 × NK603 for humans, animal or the environment.

Study identification	Title
PHI-2004-094	Evaluation of Cold Tolerance of 1507 × NK603 Maize Seedlings
PHI-2005-026	Sample Generation of Hybrid Maize Lines Containing cry34Ab1, cry35Ab1, cry1F, pat, and cp4 epsps Genes: Greenhouse Location
PHI-2005-042	Quantitative ELISA Characterization of Hybrid Maize Lines Containing cry34Ab1, cry35Ab1, cry1F, pat, and/or cp4 epsps Genes (Greenhouse)
PHI-2008-077	Agronomic Characteristics and Nutrient Composition of a Maize Line Containing the Combined Trait Product DAS-Ø15Ø7-1 × MON-ØØ81Ø-6 × MON-ØØ6Ø3-6: U.S. Test Sites
PHI-2008-103	Molecular Characterization of DAS-Ø15Ø7-1 × MON-ØØ81Ø-6 × MON-ØØ6Ø3-6 Maize Using Southern Blot Analysis and Event-Specific Polymerase Chain Reaction
PHI-2010-061	Agronomic Characteristics, Expressed Trait Protein Concentration, and Nutrient Composition of Maize Lines Containing Event DAS-Ø15Ø7-1, MON-ØØ81Ø-6, and the Combined Trait Product DAS-Ø15Ø7-1 × MON-ØØ81Ø-6: US and Canada Test Sites
PHI-2011-013	Expressed Trait Protein Concentration of a Maize Line Containing Events DAS-Ø15Ø7-1, MON-ØØ81Ø-6, SYN-IR162-4, MON-ØØ6Ø3-6 and the Combined Trait Product DAS-Ø15Ø7-1 × MON-ØØ81Ø-6 × SYN-IR162-4 × MON-ØØ6Ø3-6: US Test Sites
PHI-2011-014	Agronomic Characteristics and Nutrient Composition of a Maize Line Containing Events DAS-Ø15Ø7-1, MON-ØØ81Ø-6, SYN-IR162-4, MON-ØØ6Ø3-6, and the Combined Trait Product DAS-Ø15Ø7-1 × MON-ØØ81Ø-6 × SYN-IR162-4 × MON-ØØ6Ø3-6: US Test Sites
PHI-2011-094	Expressed Trait Protein Concentration of a Maize Line Containing Events DAS-Ø15Ø7-1, SYN-IR162-4, and the Combined Trait Product DAS-Ø15Ø7-1 × SYN-IR162-4: US Test Sites
PHI-2011-118	Expressed Trait Protein Concentration of a Maize Line Containing the Combined Trait Product DAS-Ø15Ø7-1 × MON-ØØ81Ø-6 × SYN-IR162-4 × MON-ØØ6Ø3-6: Chile Test Sites
PHI-2011-119	Agronomic Characteristics and Nutrient Composition of a Maize Line Containing the Combined Trait Product DAS-Ø15Ø7-1 × MON-ØØ81Ø-6 × SYN-IR162-4 × MON-ØØ6Ø3-6: Chile Test Sites
PHI-2012-023/011	Expressed Trait Protein Concentration of a Maize Line Containing the Combined Trait Product DAS-Ø15Ø7-1 × MON-ØØ81Ø-6 × SYN-IR162-4: U.S. and Canada Test Sites
PHI-2012-164/701	Southern Blot Analysis of Maize Hybrid XY696 Lines Containing Events DAS-Ø15Ø7-1, MON-ØØ81Ø-6, and MON-ØØ6Ø3-6
PHI-2012-185	Expressed Trait Protein Concentration in Leaf, Tassel, Silk, Pollen, Husk, and Grain Tissue of Three Hybrid Maize Lines XYG35H, XYG35YH, and XYG35YHR (Greenhouse)
PHI-2012-186	Expressed Trait Protein Concentration in Leaf, Tassel, Silk, Pollen, Husk, and Grain Tissue of Two Hybrid Maize Lines XYA91H and XYA91YH (Greenhouse)
PHI-2012-194	Agronomic Characteristics, Expressed Trait Protein Concentration, and Nutrient Composition of Maize Lines Containing the Combined Trait Product DAS-Ø15Ø7-1 × MON-ØØ81Ø-6 × SYN-IR162-4 and DAS-Ø15Ø7-1 × MON-ØØ81Ø-6 × SYN-IR162-4 × MON-ØØ6Ø3-6: Brazil Test Sites
PHI-2012-287	Six Week Poultry Feeding Study with Grains from Maize Hybrid Containing the Combined Trait Product TC1507 × NK603
PHI-2013-019	Expressed Trait Protein Concentration of a Maize Line Containing the Combined Trait Product DAS-Ø15Ø7-1 × MON-ØØ81Ø-6 × SYN-IR162-4: United States Test Sites
PHI-2013-020	Agronomic Characteristics and Nutrient Composition of a Maize Line Containing the Combined Trait Product DAS-Ø15Ø7-1 × MON-ØØ81Ø-6 × SYN-IR162-4: United States Test Sites
PHI-2013-121	Genetic Stability and Equivalency of DAS-Ø15Ø7-1 × MON-ØØ81Ø-6 × SYN-IR162-4 Maize Using Southern Blot Analysis and Event-Specific Polymerase Chain Reaction
PHI-2013-167	Rodent Diet Formulation Study Using Maize Grains Containing the Combined Trait Product TC1507 × NK603, TC1507 × MON810 × NK603 and Non-transgenic Maize Grains

Study identification	Title
PHI-2014-162	Thirteen week rat feeding study with maize grain containing the combined trait products TC1507 × NK603 and TC1507 × MON810 × NK603 (guidelines Ministry of Science and Technology Government of India, 2008)
PHI-2013-175	Agronomic Characteristics, Expressed Trait Protein Concentration, and Nutrient Composition of a Maize Line Containing the Combined Trait Product DAS-Ø15Ø7-1 × SYN-IR162-4 × MON-ØØ6Ø3-6: Brazil Test Sites
PHI-2014-064	Evaluation of Germination and Viability of a Maize Line Containing the Combined Trait Product DAS-Ø15Ø7-1 × MON-ØØ81Ø-6 × SYN-IR162-4: Controlled Environment Test Site
PHI-2014-098	Genetic Stability and Equivalency of DAS–Ø15Ø7–1 × MON–ØØ81Ø-6 Maize Using Southern Blot Analysis and Event-Specific Polymerase Chain Reaction
PHI-2014-107	Evaluation of Germination and Viability of a Maize Line Containing the Combined Trait Product DAS-Ø15Ø7-1 × MON-ØØ81Ø-6: Controlled Environment Test Site
PHI-2014-109	Genetic Stability and Equivalency of DAS-Ø15Ø7-1 × SYN-IR162-4 Maize Using Southern Blot Analysis and Event-specific Polymerase Chain Reaction
PHI-2014-110	Genetic Stability and Equivalency of DAS-Ø15Ø7-1 × SYN-IR162-4 × MON-ØØ6Ø3-6 Maize Using Southern Blot Analysis and Event-Specific Polymerase Chain Reaction
PHI-2014-131	Analysis of Single (HX1) and Triple Stack Maize (HX1 × MON810 × NK603) Samples of Spring 2014 from Various Trial Locations of Pakistan for Quantification of Cry1F, Cry1Ab and CP4 EPSPS Proteins by ELISA
PHI-2015-007/001	Evaluation of Agronomic Characteristics and Yield for a Maize Line Containing the Combined Trait Product DAS-Ø15Ø7-1 × MON-ØØ81Ø-6 × SYN-IR162-4 × MON-ØØ6Ø3-6
PHI-2014-046	Yield Characteristics of a Maize Line Containing the Combined Trait Product DAS-Ø15Ø7-1 × MON-ØØ81Ø-6 × SYN-IR162-4: U.S. Test Sites
PHI-2012-023_020	Agronomic Characteristics, Expressed Trait Protein Concentration, and Nutrient Composition of a Maize Line Containing the Combined Trait Product DAS-Ø15Ø7-1 × MON-ØØ81Ø-6 × SYN-IR162-4 × MON-ØØ6Ø3-6: U.S. and Canada Test Sites (EU Study Format)
PHI 2012-012	Nutritional Equivalency Study of the Combined Trait Product DAS-01507-1 × MON-00810-6 × SYN-1R162-4 × MON-00603-6 Poultry Feeding Study

Appendix B – Protein expression data

Mean, standard deviation and range of protein levels (μg/g dry weight) from maize 1507 × MON810 × MIR162 × NK603, 1507, MON810, MIR162 and NK603, not treated with intended herbicides, from field trials performed across four locations in USA in 2012 (n = 16[30]).

Protein	Event(s)	Leaf (V6)	Root (V9)	Leaf (V9)	Whole plant (V9)	Root (R1)	Leaf (R1)	Pollen (R1)	Stalk (R1)	Whole plant (R1)	Root (R4)	Leaf (R4)	Forage (R4)	Root (R6)	Leaf (R6)	Whole plant (R6)	Grain (R6)
Cry1F	**1507 × MON810 × MIR162 × NK603**	32[a] ± 9.9[b] (22–55)[c]	12 ± 2.8 (6.9–16)	18 ± 6.1 (14–33)	27 ± 5.2 (20–34)	10 ± 3.4 (6.6–16)	18 ± 3.3 (13–25)	31 ± 3.9 (28–42)	12 ± 1.5 (9.4–15)	18 ± 3.4 (13–26)	8.5 ± 2.3 (4.2–12)	46 ± 12 (25–60)	13 ± 1.3 (11–16)	8.7 ± 3.5 (0.48–14)	8.2 ± 9.9 (< 0.14–31)	13 ± 4.0 (6.2–20)	2.9 ± 0.80 (1.9–4.2)
	1507	21 ± 3.8 (14–26)	8.9 ± 2.0 (4.8–12)	16 ± 4.3 (9.6–24)	18 ± 2.4 (14–22)	7.2 ± 1.7 (4.5–11)	17 ± 4.9 (10–26)	26 ± 2.1 (22–30)	9.2 ± 0.77 (8.0–11)	15 ± 1.5 (13–18)	5.8 ± 2.4 (1.7–11)	23 ± 16 (1.0–41)	11 ± 1.7 (8.6–15)	6.3 ± 1.7 (2.8–9.0)	11 ± 12 (0.17–37)	9.2 ± 3.3 (5.2–17)	2.4 ± 0.61 (1.5–3.6)
PAT	**1507 × MON810 × MIR162 × NK603**	6.5 ± 2.2 (3.4–12)	0.42 ± 0.17 (0.16–0.69)	3.7 ± 0.93 (2.0–5.2)	4.7 ± 1.4 (2.6–7.6)	0.37 ± 0.14 (0.19–0.60)	4.2 ± 1.1 (2.3–7.2)	< LOQ[d]	0.042 ± 0.029 (< 0.046–0.13)	3.2 ± 1.2 (1.7–5.0)	0.36 ± 0.19 (< 0.069–0.60)	2.6 ± 1.1 (0.72–4.3)	0.92 ± 0.29 (0.5–1.5)	0.28 ± 0.18 (< 0.069–0.60)	0.15 ± 0.18 (< 0.14–0.60)	0.22 ± 0.16 (< 0.046–0.56)	< LOQ
	1507	6.9 ± 1.8 (4.4–10)	0.40 ± 0.22 (0.11– 0.84)	5.7 ± 1.5 (2.8–7.2)	5.6 ± 1.7 (3.0–8.0)	0.33 ± 0.13 (0.13–0.54)	6.0 ± 2.2 (2.8–11)	< 0.28 < 0.28	0.058 ± 0.088 (< 0.046–0.38)	3.7 ± 0.71 (2.6–5.0)	0.31 ± 0.21 (0.093–0.72)	3.4 ± 1.5 (0.96–5.8)	1.1 ± 0.36 (0.58–1.7)	0.29 ± 0.17 (< 0.069–0.57)	0.50 ± 0.73 (< 0.14–2.0)	0.32 ± 0.29 (< 0.046–0.84)	< LOQ
Cry1Ab	**1507 × MON810 × MIR162 × NK603**	35 ± 9.3 (25–52)	9.3 ± 1.8 (6.6–13)	17 ± 3.1 (14–23)	20 ± 3.5 (16–26)	8.3 ± 2.9 (5.4–14)	15 ± 2.4 (12–20)	< LOQ	5.5 ± 1.0 (4.2–7.2)	12 ± 2.4 (8.8–16)	7.4 ± 2.7 (2.4–12)	18 ± 6.1 (7.2–24)	6.2 ± 1.0 (4.2–8.6)	8.0 ± 4.2 (0.36–14)	5.0 ± 5.3 (0.72–17)	5.8 ± 2.7 (1.5–10)	0.21 ± 0.062 (0.12–0.30)
	MON810	37 ± 8.1 (28–56)	8.8 ± 2.1 (3.0–11)	21 ± 4.2 (12–30)	22 ± 4.6 (17–30)	8.2 ± 2.8 (4.8–14)	19 ± 4.8 (11–26)	< LOQ	5.7 ± 0.98 (4.6–7.6)	14 ± 2.4 (10–18)	6.2 ± 3.7 (0.63–11)	15 ± 9.7 (1.7–28)	7.1 ± 1.7 (4.8–9.6)	10 ± 5.5 (0.99–19)	7.1 ± 8.3 (0.59–23)	6.4 ± 3.1 (1.7–12)	0.20 ± 0.074 (0.13–0.42)
Vip3Aa20	**1507 × MON810 × MIR162 × NK603**	160 ± 72 (78–310)	48 ± 14 (20–75)	99 ± 29 (66–170)	170 ± 27 (140–220)	34 ± 13 (13–54)	130 ± 35 (66–180)	150 ± 15 (120–170)	60 ± 14 (40–92)	140 ± 24 (110–190)	32 ± 13 (13–51)	400 ± 140 (190–660)	230 ± 38 (170–320)	55 ± 32 (0.87–99)	110 ± 150 (0.59–500)	130 ± 57 (48–260)	100 ± 80 (14–280)
	MIR162	120 ± 37 (66–170)	38 ± 9.4 (23–51)	96 ± 29 (60–170)	160 ± 31 (110–200)	29 ± 9.0 (15–45)	160 ± 57 (54–220)	130 ± 13 (100–140)	73 ± 11 (58–98)	150 ± 28 (100–200)	26 ± 13 (7.8–51)	360 ± 110 (140–530)	190 ± 74 (130–440)	52 ± 28 (1.7–87)	160 ± 200 (< LOQ–600)	180 ± 110 (22–400)	110 ± 41 (45–180)

[30] Except the following tissues, for which n = 15: Root (V9) of MON810, MIR162 and NK603, for all proteins analysed for each event; Root (R1) of 1507 × MON810 × MIR162 × NK603, for all analysed proteins.

Protein	Event(s)	Leaf (V6)	Root (V9)	Leaf (V9)	Whole plant (V9)	Root (R1)	Leaf (R1)	Pollen (R1)	Stalk (R1)	Whole plant (R1)	Root (R4)	Leaf (R4)	Forage (R4)	Root (R6)	Leaf (R6)	Whole plant (R6)	Grain (R6)
PMI	**1507 × MON810 × MIR162 × NK603**	8.5 ± 3.4 (4.4–16)	2.9 ± 0.87 (1.3–4.5)	5.0 ± 1.4 (3.7–8.4)	5.9 ± 1.0 (4.6–7.6)	2.0 ± 0.72 (1.1–3.3)	5.1 ± 0.76 (3.6–6.6)	4.6 ± 0.40 (4.0–5.3)	3.0 ± 0.78 (2.2–4.4)	4.8 ± 0.66 (3.8–5.6)	1.4 ± 0.58 (0.51–2.3)	10 ± 2.7 (5.0–14)	4.9 ± 0.68 (3.6–6.2)	2.7 ± 1.6 (< LOQ–5.1)	1.7 ± 2.5 (< LOQ–9.0)	3.7 ± 1.7 (0.54–7.4)	1.6 ± 0.63 (0.81–2.7)
	MIR162	7.2 ± 2.0 (4.3–9.6)	2.7 ± 0.69 (1.6–3.9)	5.1 ± 1.2 (3.7–7.8)	5.7 ± 1.1 (3.8–7.2)	1.7 ± 0.68 (0.93–3.3)	6.4 ± 1.2 (4.0–7.8)	4.6 ± 0.57 (3.8–5.9)	3.3 ± 0.71 (2.2–4.6)	4.8 ± 0.73 (3.8–6.2)	0.93 ± 0.64 (< LOQ–2.0)	11 ± 3.4 (5.0–16)	5.0 ± 1.3 (4.0–9.2)	2.6 ± 1.4 (< LOQ–4.2)	3.0 ± 4.1 (< LOQ–13)	3.6 ± 2.4 (0.60–9.0)	1.5 ± 0.45 (0.84–2.2)
CP4 EPSPS	**1507 × MON810 × MIR162 × NK603**	230 ± 65 (140–400)	110 ± 44 (36–160)	160 ± 36 (110–230)	260 ± 60 (160–440)	93 ± 31 (57–140)	170 ± 19 (140–210)	460 ± 78 (250–580)	110 ± 35 (76–180)	180 ± 29 (130–220)	75 ± 12 (51–100)	340 ± 100 (160–500)	120 ± 24 (86–180)	79 ± 41 (0.51–150)	78 ± 94 (< LOQ–250)	110 ± 54 (19–220)	11 ± 3.7 (6.0–17)
	NK603	230 ± 46 (170–330)	79 ± 13 (51–96)	190 ± 22 (140–220)	260 ± 35 (200–340)	87 ± 32 (36–140)	270 ± 69 (180–480)	400 ± 84 (200–520)	130 ± 45 (80–220)	250 ± 46 (170–320)	56 ± 19 (36–110)	370 ± 140 (140–660)	110 ± 28 (78–170)	97 ± 43 (19–160)	100 ± 110 (< LOQ–320)	120 ± 57 (38–220)	8.5 ± 2.3 (5.4–13)

(a): Mean.
(b): Standard deviation.
(c): Range.
(d): LOQ – limit of quantification.

Appendix C – List of relevant publications

List of relevant publications identified by the applicant **through systematic literature searches (January 2005–May 2020)**

References
Bowers E, Munkvold G and Hellmich RL, 2013. Vip3Aa and Cry1Ab proteins in maize reduce *Fusarium* ear rot and fumonisins by deterring kernel injury from multiple Lepidopteran pests. World Mycotoxin Journal, 6, 127–135.
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Graser G, Walters FS, Burns A, Sauve A and Raybould A, 2017. A general approach to test for interaction among mixtures of insecticidal proteins which target different orders of insect pests. Journal of Insect Science, 17, 1–12.
Park KW, Lee B, Kim C-G and Kim D-Y, 2010. Monitoring the occurrence of genetically modified maize at a grain receiving port and along transportation routes in the Republic of Korea. Food Control, 21, 456–461.
Ridley W, Harrigan GG, Breeze ML, Nemeth MA, Sidhu RS and Glenn KC, 2011. Evaluation of compositional equivalence for multitrait biotechnology crops. Journal of Agricultural and Food Chemistry, 59, 5865–5876.
Waminal NE, Ryu KH, Choi SH and Kim HH, 2013. Randomly detected genetically modified (GM) maize (*Zea mays* L.) near a transport route revealed a fragile 45S rDNA phenotype. PLoS ONE, 8, e74060.

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

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

Appendix E – Animal dietary exposure

Animal dietary exposure to Cry1F, PAT, Cry1Ab, Vip3Aa20, PMI and CP4 EPSPS proteins (mg/kg bw per day) in livestock, based on the consumption of maize grain and forage.

	Dietary exposure (mg/kg bw per day)					
	Cry1F			**PAT**		
	Grain (G)	**Forage (F)**	**G + F**	**Grain (G)**	**Forage (F)**	**G + F**
Broiler	0.16	NA	NA	0.0034	NA	NA
Layer	0.15	NA	NA	0.0033	NA	NA
Turkey	0.11	NA	NA	0.0025	NA	NA
Breeding pigs	0.05	NA	NA	0.0011	NA	NA
Finishing pigs	0.07	NA	NA	0.0014	NA	NA
Beef cattle	0.06	0.27	0.33	0.0013	0.021	0.022
Dairy cattle	0.04	0.32	0.36	0.0008	0.025	0.026
Ram/ewe	0.03	NA	NA	0.0007	NA	NA
Lamb	0.04	0.18	0.22	0.0009	0.014	0.015
	Dietary exposure (mg/kg bw per day)					
	Cry1Ab			Vip3Aa20		
	Grain (G)	Forage (F)	G + F	Grain (G)	Forage (F)	G + F
Broiler	0.009	NA	NA	2.42	NA	NA
Layer	0.009	NA	NA	2.35	NA	NA
Turkey	0.006	NA	NA	1.75	NA	NA
Breeding pigs	0.003	NA	NA	0.79	NA	NA
Finishing pigs	0.004	NA	NA	1.03	NA	NA
Beef cattle	0.004	0.14	0.14	0.94	4.80	5.74
Dairy cattle	0.002	0.16	0.17	0.57	5.77	6.33
Ram/ewe	0.002	NA	NA	0.49	NA	NA
Lamb	0.002	0.09	0.09	0.62	3.19	3.81
	Dietary exposure (mg/kg bw per day)					
	PMI			CP4 EPSPS		
	Grain (G)	Forage (F)	G + F	Grain (G)	Forage (F)	G + F
Broiler	0.079	NA	NA	0.59	NA	NA
Layer	0.077	NA	NA	0.57	NA	NA
Turkey	0.057	NA	NA	0.43	NA	NA
Breeding pigs	0.026	NA	NA	0.19	NA	NA
Finishing pigs	0.034	NA	NA	0.25	NA	NA
Beef cattle	0.031	0.10	0.13	0.23	2.7	2.9
Dairy cattle	0.018	0.12	0.14	0.14	3.2	3.4
Ram/ewe	0.016	NA	NA	0.12	NA	NA
Lamb	0.020	0.07	0.09	0.15	1.8	1.9