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Assessment of genetically modified maize Bt11 x MIR162 x 1507 x GA21 and three subcombinations independently of their origin, for food and feed uses under Regulation (EC) No 1829/2003 (application EFSA-GMO-DE-2010-86)

EFSA Panel on Genetically Modified Organisms (EFSA GMO Panel), Hanspeter Naegeli, Andrew Nicholas Birch, Josep Casacuberta, Adinda De Schrijver, Mikołaj Antoni Gralak, Philippe Guerche, Huw Jones, Barbara Manachini, Antoine Messéan, Elsa Ebbesen Nielsen, Fabien Nogué, Christophe Robaglia, Nils Rostoks, Jeremy Sweet, Christoph Tebbe, Francesco Visioli, Michele Ardizzone, Silvia Federici, Antonio Fernandez Dumont, Andrea Gennaro, Jose Ángel Gómez Ruiz, Anna Lanzoni, Franco Maria Neri, Nikoletta Papadopoulou and Konstantinos Paraskevopoulos

Abstract

In this opinion, the GMO Panel assessed the four-event stack maize Bt11 × MIR162 × 1507 × GA21 and three of its subcombinations, independently of their origin. The GMO Panel previously assessed the four single events and seven of their combinations and did not identify safety concerns. No new data on the single events or the seven subcombinations leading to modification of the original conclusions were identified. Based on the molecular, agronomic, phenotypic and compositional characteristics, the combination of the single events in the four-event stack maize did not give rise to food/feed safety issues. Based on the nutritional assessment of the compositional characteristics of maize Bt11 × MIR162 × 1507 × GA21, foods and feeds derived from the genetically modified (GM) maize are expected to have the same nutritional impact as those derived from non-GM maize varieties. In the case of accidental release of viable grains of maize Bt11 × MIR162 × 1507 × GA21 into the environment, this would not raise environmental safety concerns. The GMO Panel concludes that maize Bt11 × MIR162 × 1507 × GA21 is nutritionally equivalent to and as safe as its non-GM comparator in the context of the scope of this application. For the three subcombinations included in the scope, for which no experimental data were provided, the GMO Panel assessed the likelihood of interactions among the single events and concluded that their combinations would not raise safety concerns. These maize subcombinations are therefore 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 maize Bt11 × MIR162 × 1507 × GA21 and its subcombinations. A minority opinion expressed by a GMO Panel member is appended to this opinion.

Keywords: GMO, maize (*Zea mays*), Bt11, MIR162, 1507, GA21, insect resistant and herbicide tolerant

Requestor: Competent Authority of Germany

Question number: EFSA-Q-2010-01087

Correspondence: GMO@efsa.europa.eu

Panel members: Andrew Nicholas Birch, Josep Casacuberta, Adinda De Schrijver, Mikołaj Antoni Gralak, Philippe Guerche, Huw Jones, Barbara Manachini, Antoine Messéan, Hanspeter Naegeli, Elsa Ebbesen Nielsen, Fabien Nogué, Christophe Robaglia, Nils Rostoks, Jeremy Sweet, Christoph Tebbe, Francesco Visioli and Jean-Michel Wal.

Minority opinion: This scientific opinion is not shared by the following member of the Panel: Jean-Michel Wal (see Appendix B).

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Summary

Following the submission of application EFSA–GMO–DE–2010–86 under Regulation (EC) No 1829/2003 from Syngenta, the Panel on Genetically Modified Organisms of the European Food Safety Authority (referred to hereafter as the GMO Panel) was asked to deliver a scientific opinion on the safety of genetically modified (GM) herbicide-tolerant and insect-resistant maize Bt11 × MIR162 × 1507 × GA21 (referred to hereafter as 'four-event stack maize') and its subcombinations independently of their origin (referred to hereafter as 'subcombinations'). The scope of application EFSA-GMO-DE-2010-86, which included all subcombinations at the time of submission, was subsequently limited to include three subcombinations only. The scope of application EFSA-GMO-DE-2010-86 as assessed in this Scientific Opinion is for the placing on the market of maize Bt11 × MIR162 × 1507 × GA21 and three subcombinations (Bt11 × MIR162 × 1507, MIR162 × 1507 × GA21 and MIR162 × 1507), independently of their origin, for food and feed uses, import and processing.

The term 'subcombination' refers to any combination of up to three of the events present in the four-event stack maize Bt11 × MIR162 × 1507 × GA21. In the present GMO Panel Scientific Opinion, the safety of subcombinations occurring as segregating progeny in the harvested grains of maize Bt11 × MIR162 × 1507 × GA21 is evaluated in the context of the assessment of the four-event stack maize. The safety of subcombinations that either have 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 maize, is assessed in a separate section of the Scientific Opinion.

In delivering its Scientific Opinion, the GMO Panel considered the data available on the single events, the four-event stack maize, the previously assessed subcombinations, the scientific comments submitted by the Member States and relevant scientific literature. The four-event stack maize Bt11 × MIR162 × 1507 × GA21 was produced by conventional crossing to combine four single maize events: Bt11, expressing the Cry1Ab protein for protection against some lepidopteran pests and the phosphinothricin acetyltransferase (PAT) protein for tolerance to glufosinate-ammonium-containing herbicides; MIR162, expressing the Vip3Aa20 protein for protection against some lepidopteran pests and the phosphomannose isomerase (PMI) protein used as a selectable marker; 1507, expressing the Cry1F protein for protection against some lepidopteran pests and the PAT protein for tolerance to glufosinate-ammonium-containing herbicides; and GA21, expressing the mutated 5-enolpyruvyl-shikimate-3-phosphate synthase (mEPSPS) protein for tolerance to glyphosate-containing herbicides.

The GMO Panel evaluated the four-event stack maize and three of its subcombinations with reference to the scope and appropriate principles described in its guidelines for the risk assessment of GM plants and derived food and feed, the environmental risk assessment of GM plants and the post-market environmental monitoring of GM plants. The GMO Panel Guidance Documents establish the principle that where all single events have been assessed, the risk assessment of stacked events should focus mainly on issues related to (a) stability of the inserts, (b) expression of the introduced genes and their products and (c) potential synergistic or antagonistic effects resulting from the combination of the events.

For application EFSA-GMO-DE-2010-86, the previous assessments of the four single maize events and seven subcombinations provided a basis to evaluate the four-event stack maize and the three subcombinations included in the scope of the application. The four single maize events (Bt11, MIR162, 1507 and GA21) and seven subcombinations (Bt11 × GA21 × MIR162, Bt11 × 1507 × GA21, Bt11 × GA21, MIR162 × GA21, Bt11 × MIR162, Bt11 × 1507 and 1507 × GA21) were previously assessed by the GMO Panel and no concerns on their safety were identified. 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 analyses of agronomic/phenotypic and compositional characteristics was undertaken, and the safety of the newly expressed proteins and the whole food/feed were evaluated with respect to potential toxicity, allergenicity and nutritional characteristics. An evaluation of environmental impacts and post-market environmental monitoring plans was also undertaken.

The molecular data establish that the events stacked in maize Bt11 × MIR162 × 1507 × GA21 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 except for the

expected difference for PAT protein levels resulting from the combination of Bt11 and 1507 events, both producing PAT protein in the four-event stack. 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/phenotypic characteristics identified no differences between maize Bt11 × MIR162 × 1507 × GA21 and the non-GM comparator that required further assessment for food/feed safety or environmental impact, except for a decrease of β-carotene, β-cryptoxanthin and lutein in GM maize grain.

The combination of maize Bt11, MIR162, 1507 and GA21 and of the newly expressed proteins in the four-event stack maize did not give rise to issues regarding food and feed safety. Based on the nutritional assessment of the levels of β-carotene, β-cryptoxanthin and lutein in the GM maize, the GMO Panel concludes that foods and feeds derived from maize Bt11 × MIR162 × 1507 × GA21 are expected to have the same nutritional impact as those derived from non-GM commercial maize varieties.

Considering the combined events, the outcome of the comparative analysis and the routes and levels of exposure, the GMO Panel concludes that this four-event stack maize would not raise safety concerns in the case of accidental release of viable GM maize grains into the environment.

The GMO Panel concludes that the four-event stack maize is as safe as the non-GM comparator with respect to potential effects on human and animal health and the environment in the context of the scope of this application.

Maize Bt11 × MIR162 × 1507 × GA21 has 10 possible subcombinations, seven of which have been previously assessed and are not included in the scope of application EFSA-GMO-DE-2010-86. The three remaining subcombinations (Bt11 × MIR162 × 1507, MIR162 × 1507 × GA21 and MIR162 × 1507) were not previously assessed and are included in the scope of the application. For these subcombinations, for which no experimental data were provided, the GMO Panel assessed the possibility of interactions between the events and concluded that these combinations of maize events Bt11, MIR162, 1507 and GA21 would not raise safety concerns. The three maize subcombinations are therefore expected to be as safe as the single events, the previously assessed subcombinations and the four-event stack maize Bt11 × MIR162 × 1507 × GA21.

Given the absence of safety concerns for food and feed derived from maize Bt11 × MIR162 × 1507 × GA21 and its subcombinations, the GMO Panel considers that post-market monitoring of these products is not necessary. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of the four-event stack maize and its subcombinations.

A minority opinion expressed by a GMO Panel member is presented in Appendix B of this opinion.

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1. Introduction

1.1. Background

On 10 August 2010, the European Food Safety Authority (EFSA) received from the Competent Authority of Germany application EFSA-GMO-DE-2010-86, for authorisation of GM maize Bt11 × MIR162 × 1507 × GA21 submitted by Syngenta (referred to hereafter as 'the applicant') within the framework of Regulation (EC) No 1829/2003[1] on GM food and feed for food and feed uses, import and processing.

After receiving application EFSA-GMO-DE-2010-86 and in accordance with Articles 5(2)(b) and 17(2)(b) of Regulation (EC) No 1829/2003, EFSA informed Member States and the European Commission, and made the summary of the application available to the public on the EFSA website.[2] EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of Regulation (EC) No 1829/2003. On 26 January 2011, 25 March 2011, 21 December 2011 and 2 April 2012, EFSA received additional information under completeness check (requested on 11 October 2010, 4 March 2011 and 25 January 2012, respectively). On 14 June 2012, EFSA declared the application valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the European Commission and consulted nominated risk assessment bodies of Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC[3] following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003 to request their scientific opinion. Member States had 3 months after the date of receipt of the valid application (until 18 September 2012) to make their opinion known.

The scope defined by the applicant at the time of submission was 'all food and feed products containing, consisting or produced from Bt11 × MIR162 × 1507 × GA21 maize including products from inbreds and hybrids obtained by conventional breeding of this stacked maize product. The application also covers the import and industrial processing of Bt11 × MIR162 × 1507 × GA21 maize for all potential uses as any other maize.' After clarifications (letters dated 15 March 2012, 6 June 2012, 18 February 2014, 8 December 2015 and 31 March 2016), the applicant notified EFSA that the scope of application EFSA-GMO-DE-2010-86 was limited to 'Bt11 × MIR162 × 1507 × GA21 maize and three subcombinations from Bt11 × MIR162 × 1507 × GA21 maize (Bt11 × MIR162 × 1507, MIR162 × 1507 × GA21 and MIR162 × 1507) independently of their origin.'

The genetically modified organism (GMO) Panel carried out an evaluation of the scientific risk assessment of GM maize Bt11 × MIR162 × 1507 × GA21 and the three subcombinations listed above. The GMO Panel requested additional information from the applicant on 31 October 2012 (EURL-GMFF), 7 December 2012, 5 February 2013, 27 September 2013, 4 June 2014, 16 September 2014, 3 October 2014, 10 November 2014, 22 September 2015, 23 December 2015, 4 August 2016, 1 February 2017, 18 May 2017, 20 December 2017, 18 January 2018 and 16 February 2018. The applicant provided the requested information on 16 November 2012 (EURL-GMFF), 26 April 2013, 16 June 2014, 23 June 2014, 30 September 2014, 21 November 2014, 28 November 2014, 12 March 2015, 30 July 2015, 10 August 2015, 9 September 2015, 24 September 2015, 4 April 2016, 3 November 2016, 12 June 2017, 14 December 2017, 5 March 2018, 5 April 2018 and 30 April 2018. The applicant also provided spontaneously additional information on 28 July 2014, 21 July 2015, 17 December 2015, 25 November 2016 and 4 October 2017.

In the context of contract OC/EFSA/UNIT/GMO/2013/01, the contractors performed preparatory work and delivered reports on the methods applied by the applicant in performing bioinformatic analyses.

In giving its scientific opinion to the European Commission, the Member States and the applicant, and in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003 (European Commission, 2003), EFSA has endeavoured to respect a time limit of 6 months from the acknowledgement of the valid application. As additional information was requested by the GMO Panel, the time limit of 6 months was extended accordingly, in line with Articles 6(1), 6(2), 18(1) and 18(2) of Regulation (EC) No 1829/2003.

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. Official Journal of the European Communities, L268, 1–23.
[2] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2010-01087
[3] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 12.3.2001, p. 1–38.

According to Regulation (EC) No 1829/2003 (European Commission, 2003), this scientific opinion is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation and thus will be part of the EFSA overall opinion in accordance with Articles 6(5) and 18(5).

1.2. Terms of Reference as provided by the requestor

The GMO Panel was requested to carry out a scientific risk assessment of 'Bt11 × MIR162 × 1507 × GA21 maize and three subcombinations from Bt11 × MIR162 × 1507 × GA21 maize (Bt11 × MIR162 × 1507, MIR162 × 1507 × GA21 and MIR162 × 1507) independently of their origin', for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003.

Where applicable, any conditions or restrictions which should be imposed on the placing on the market and/or specific conditions or restrictions for use and handling, including post-market monitoring requirements based on the outcome of the risk assessment and, in the case of GMOs or food/feed containing or consisting of GMOs, conditions for the protection of particular ecosystems/environment and/or geographical areas should be indicated in accordance with Articles 6(5)(e) and 18(5)(e) of Regulation (EC) No 1829/2003.

The GMO Panel was not requested to give an opinion on information required under Annex II to the Cartagena Protocol. Furthermore, the GMO Panel did not consider proposals for labelling and methods of detection (including sampling and the identification of the specific transformation event in the food/feed and/or food/feed produced from it), which are matters related to risk management.

2. Data and methodologies

2.1. Data

In delivering its scientific opinion, the GMO Panel took into account application EFSA-GMO-DE-2010-86, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications.

2.2. Methodologies

The GMO Panel carried out a scientific risk assessment of maize Bt11 × MIR162 × 1507 × GA21 and three subcombinations that have not been authorised previously (Table 1), independently of their origin, for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. The GMO Panel took into account the appropriate principles described in its guidelines for the risk assessment of GM plants and derived food and feed (EFSA, 2006, 2007a; EFSA GMO Panel, 2011a), for the environmental risk assessment (ERA) of GM plants (EFSA GMO Panel, 2010a) and for the post-market environmental monitoring (PMEM) of GM plants (EFSA GMO Panel, 2011b).

The comments raised by Member States are addressed in Annex G of EFSA's overall opinion and were taken into consideration during the scientific risk assessment.[2]

3. Assessment

3.1. Introduction

Application EFSA-GMO-DE-2010-86 covers four events: the four-event stack maize Bt11 × MIR162 × 1507 × GA21 and three subcombinations that have not been authorised previously, independently of their origin (Table 1). The scope of this application is for food and feed uses, import and processing, and excludes cultivation within the European Union (EU).

The term 'subcombination' refers to combinations of up to three of the events present in the four-event stack maize.

The safety of subcombinations occurring as segregating progeny in harvested grains of maize Bt11 × MIR162 × 1507 × GA21 is evaluated in the context of the assessment of the four-event stack maize in Section 3.3 of this GMO Panel Scientific Opinion.

'Subcombination' also covers combinations of up to three of the events Bt11, MIR162, 1507 and GA21 that either have 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. Seven of these subcombinations have already been assessed by the GMO Panel. The three remaining subcombinations are assessed in Section 3.4 of this GMO Panel Scientific Opinion.

The four-event stack maize was produced by conventional crossing to combine four single maize events: Bt11 (expressing the Cry1Ab and phosphinothricin acetyltransferase (PAT) proteins); MIR162 (expressing the Vip3Aa20 and phosphomannose isomerase (PMI) proteins); 1507 (expressing the Cry1F and PAT proteins); and GA21 (expressing the mutated 5-enolpyruvyl-shikimate-3-phosphate synthase (mEPSPS) protein).

Herbicide tolerance traits are achieved by the expression of the PAT and mEPSPS proteins (for tolerance to glufosinate-ammonium- and glyphosate-containing herbicides, respectively). Insect resistance traits are achieved by the expression of the Cry1Ab, Cry1F and Vip3Aa20 proteins, which confer protection against specific lepidopteran pests, such as *Ostrinia nubilalis* (European corn borer) and *Sesamia nonagrioides* (Mediterranean corn borer).

Table 1: The four maize events covered by the scope of application EFSA-GMO-DE-2011-86

Degree of stacking	Events	Unique Identifiers
Four-event stack maize	Bt11 × MIR162 × 1507 × GA21	SYN-BTØ11-1 x SYN-IR162-4 x DAS-Ø15Ø7-1 x MON-ØØØ21-9
Three-event stack maize	Bt11 × MIR162 × 1507	SYN-BTØ11-1 x SYN-IR162-4 x DAS-Ø15Ø7-1
	MIR162 × 1507 × GA21	SYN-IR162-4 x DAS-Ø15Ø7-1 x MON-ØØØ21-9
Two-event stack maize	MIR162 × 1507	SYN-IR162-4 x DAS-Ø15Ø7-1

The four single maize events and seven subcombinations have been previously assessed by the GMO Panel (Table 2), and no safety concerns were identified.

Table 2: Single maize events and subcombinations of maize Bt11 × MIR162 × 1507 × GA21 already assessed by the GMO Panel

Events	Application or mandate	Reference
Bt11	C/F/96/05.10 EFSA-GMO-RX-Bt11 EFSA-M-2012-0232[(a)]	EFSA (2005a) EFSA (2009a) EFSA GMO Panel (2012a)
MIR162	EFSA-GMO-DE-2010-82	EFSA GMO Panel (2012b)
1507	C/NL/00/10 C/ES/01/0 EFSA-GMO-UK-2004-02 EFSA-GMO-RX-1507 EFSA-M-2012-0231[(b)] EFSA-GMO-RX-001	EFSA (2004) EFSA (2005b) EFSA (2005c) EFSA (2009b) EFSA GMO Panel (2012c) EFSA GMO Panel (2017a)
GA21	EFSA-GMO-UK-2005-19 EFSA-GMO-RX-GA21 EFSA-GMO-RX-005	EFSA (2007b) EFSA (2007b) EFSA GMO Panel (2017b)
Bt11 x GA21	EFSA-GMO-UK-2007-49	EFSA GMO Panel (2009a)
Bt11 x GA21 x MIR162	EFSA-GMO-DE-2009-66	EFSA GMO Panel (2015a)
MIR162 x GA21	EFSA-GMO-DE-2009-66	EFSA GMO Panel (2015a)
Bt11 x MIR162	EFSA-GMO-DE-2009-66 EFSA-M-2016-0248[(c)]	EFSA GMO Panel (2015a) EFSA GMO Panel (2017c)
Bt11 x 1507 x GA21	EFSA-GMO-DE-2011-99 EFSA-M-2017-0169[(d)]	EFSA GMO Panel (2016) EFSA GMO Panel (2017d)
Bt11 x 1507	EFSA-GMO-DE-2011-99	EFSA GMO Panel (2016)
1507 x GA21	EFSA-GMO-DE-2011-99	EFSA GMO Panel (2016)

(a): Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2012-00713
(b): Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2012-00712
(c): Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q- 2016-00730
(d): Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2017-00669

EFSA guidance establishes the principle that 'For GM plants containing a combination of transformation events (stacked events), the primary concern for risk assessment is to establish that the combination of events is stable and that no interactions between the stacked events, that may raise safety concerns compared to the single events, occur. The risk assessment of GM plants containing stacked events focuses on issues related to: (a) stability of the inserts, (b) expression of the introduced genes and their products and (c) potential synergistic or antagonistic effects resulting from the combination of the events' (EFSA, 2007a; EFSA GMO Panel, 2011a).

3.2. Updated information on the events

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

For maize event GA21, updated nucleotide sequence information was received.[4] The new sequence information revealed a nucleotide change in the actin promoter of copy 6, a three-base pair deletion contiguous to one nucleotide substitution within the 3' insert flanking region, and a difference in the number of complete m*epsps* cassettes present within the insert, with respect to the sequence submitted in the original application (EFSA, 2007b). Further analyses demonstrated that these differences were already present in the original material used for the risk assessment of maize GA21. The GMO Panel has performed the risk assessment of the new sequencing information for maize event GA21 in the frame of a request received from the European Commission[5] and has concluded that the original risk assessments of maize event GA21 as a single and as a part of stacked events remain valid (EFSA GMO Panel, 2015b).

In addition, the applicant clarified that the 1507 maize sequence reported for the four-event stack maize contained one nucleotide change in the insert sequence compared to the corrected original 1507 maize sequence (EFSA GMO Panel, 2017a). Analysis of the new sequencing data and bioinformatics analyses performed on the new sequence did not identify any need for further safety assessment.[6]

Bioinformatics analyses on the junction regions for maize events Bt11, MIR162, 1507 and GA21, using the most up-to-date nucleotide sequences and methodology specified in EFSA guidance (EFSA GMO Panel, 2011a), confirmed that no known endogenous genes were disrupted by any of the inserts.[7,8]

Updated bioinformatics analyses of the amino acid sequence of the newly expressed Cry1Ab, Cry1F, Vip3Aa20, PAT, mEPSPS and PMI proteins revealed no significant similarities to toxins and allergens.[7,8,9] In addition, updated bioinformatics analyses of the newly created open-reading frames (ORFs) within the inserts and at their junctions indicated that the expression of an ORF showing significant similarities to toxins or allergens is highly unlikely.[7,8,10]

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. Risk assessment of the four-event stack maize Bt11 × MIR162 × 1507 × GA21

3.3.1. Molecular characterisation

Possible interactions that would affect the integrity of the events, newly expressed proteins levels or the biological function conferred by the individual inserts are considered.

3.3.1.1. Genetic elements and biological functions of the inserts

Maize events Bt11, MIR162, 1507 and GA21 were combined by conventional crossing to produce the four-event stack maize Bt11 × MIR162 × 1507 × GA21. The structures of the inserts introduced into the four-event stack maize are described in detail in the respective EFSA scientific opinions (Table 2) and no new genetic modifications were involved. Genetic elements in the expression cassettes of the single events are summarised in Table 3.

[4] Additional information: 21/7/2015 and 24/9/2015.
[5] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2015-00475
[6] Additional information: 24/9/2015, 12/6/2017 and 5/4/2018.
[7] Additional information: 16/6/2014, 28/7/2014 (spontaneous submission) and 30/9/2014.
[8] Additional information: 12/6/2017 and 5/4/2018.
[9] Additional information: 30/7/2015.
[10] Additional information: 30/7/2015, 17/12/2015 (spontaneous submission) and 4/4/2016.

Intended effects of the inserts in maize Bt11 × MIR162 × 1507 × GA21 are summarised in Table 4.

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

Table 3: Genetic elements in the expression cassettes of the events stacked in maize Bt11 × MIR162 × 1507 × GA21

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
Bt11	35S (CaMV)*	IVS6 (*Zea mays*)	No	*cry1Ab* (*Bacillus thuringiensis*)	*nos* (*Agrobacterium tumefaciens*)
	35S (CaMV)	IVS2 (*Zea mays*)	No	*pat* (*Streptomyces viridochromogenes*)	*nos* (*Agrobacterium tumefaciens*)
MIR162	ZmUbiInt (*Zea mays*)	–	No	*vip3Aa20* (*Bacillus thuringiensis*)	35S (CaMV)
	ZmUbiInt (*Zea mays*)	–	No	*pmi* (*Escherichia coli*)	*nos* (*Agrobacterium tumefaciens*)
1507[(a)]	*ubi*ZM1 (*Zea mays*)	–	No	*cry1F* (*Bacillus thuringiensis*)	ORF25PolyA (*Agrobacterium tumefaciens*)
	35S (CaMV)	–	No	*pat* (*Streptomyces viridochromogenes*)	35S (CaMV)
GA21	actin 1 (*Oryza sativa*)	actin 1 (*Oryza sativa*)	OTP (*Helianthus annuus*)	m*epsps* (*Zea mays*)	*nos* (*Agrobacterium tumefaciens*)

UTR: untranslated region; CaMV: cauliflower mosaic virus.
–: when no element was specifically introduced to optimise expression.
*: Source of genetic material.
(a): Maize 1507 contains additional partial fragments of the *cry1F* and *pat* genes at a single locus in the nuclear genome.

Table 4: Characteristics and intended effects of the events stacked in maize Bt11 × MIR162 × 1507 × GA21

Event	Protein	Donor organism and biological function	Intended effects in GM plant
Bt11	Cry1Ab	Donor organism: *Bacillus thuringiensis* var. *kurstaki* HD-1. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes (Schnepf et al., 1998)	Event Bt11 expresses a truncated version of the Cry1Ab protein. Cry1Ab is a protein toxic to certain lepidopteran larvae feeding on maize.
	PAT	Donor organism: *Streptomyces viridochromogenes* Tü494 Phosphinothricin-acetyl-transferase (PAT) enzyme acetylates L-glufosinate-ammonium and thereby confers tolerance to phosphinotricin (Wohlleben et al., 1988)	Expression of PAT in maize Bt11 confers tolerance to glufosinate-ammonium-based herbicides.

Event	Protein	Donor organism and biological function	Intended effects in GM plant
MIR162	Vip3Aa20	Donor organism: *B. thuringiensis* strain AB88 (Estruch et al., 1996). In addition to Cry proteins, *B. thuringiensis* also produces insecticidal proteins during its vegetative growth stage. These are referred to as vegetative insecticidal proteins (Fang et al., 2007)	Event MIR162 expresses a modified version of the *B. thuringiensis vip3Aa1* gene and encodes Vip3Aa20, a protein toxic to certain lepidopteran larvae feeding on maize
	PMI	Donor organism: *E. coli*. PMI (phosphomannose isomerase) catalyses the isomerisation of mannose-6-phosphate to fructose-6-phosphate and plays a role in the metabolism of mannose (Markovitz et al., 1967)	PMI is used as a selectable marker in maize MIR162. 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)
1507	Cry1F	Donor organism: *Bacillus thuringiensis* subsp. *aizawai*. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes (Schnepf et al., 1998).	Event 1507 expresses a truncated version of the Cry1F protein. Cry1F is a protein toxic to certain lepidopteran larvae feeding on maize.
	PAT	Donor organism: *Streptomyces viridochromogenes* strain Tü494 Phosphinothricin-acetyl-transferase (PAT) enzyme confers resistance to phosphinotricin (Thompson et al., 1987; Wohlleben et al., 1988)	PAT acetylates L-glufosinate-ammonium and thereby confers tolerance to glufosinate-ammonium-based herbicides (Droge-Laser et al., 1994)
GA21	mEPSPS	Donor organism: *Zea mays.* 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) is an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Herrmann, 1995)	The amino acid sequence of the maize EPSPS enzyme was modified to render the maize tolerant to glyphosate. Expression of mEPSPS confers tolerance to glyphosate-based herbicides (Lebrun et al., 2003)

3.3.1.2. Integrity of the events in the four-event stack[11]

The genetic stability of the inserted DNA over multiple generations in the single maize events Bt11, MIR162, 1507 and GA21 was demonstrated previously (see Table 2). Integrity of these events in maize Bt11 × MIR162 × 1507 × GA21 was demonstrated by Southern analyses.[12]

3.3.1.3. Information on the expression of the inserts[13]

Cry1Ab, PAT, Vip3Aa20, PMI, Cry1F and mEPSPS protein levels were analysed by enzyme-linked immunosorbent assay (ELISA) in material harvested from field trials at one single location in the USA[14] during the 2008 growing season. Samples analysed included leaves (V10 and R1 stages), root (R1 stage), pollen (R1 stage), whole plant (R1 stage) and grain (R6 stage) (see Appendix A). Grains and forage are the two main raw commodities used for food and feed purposes. The GMO Panel requested protein expression data from forage, but the applicant did not provide this information. In the absence of these data, the data on the highest levels of newly expressed proteins in leaves (V10 or R1 stages) were used to estimate the levels in forage. The mean values of the protein levels in grains and leaves of maize Bt11 × MIR162 × 1507 × GA21 are summarised in Table 5.

[11] Dossier: Part I – Section D5.
[12] Dossier: Part I – Section D5 and Appendix 2.
[13] Dossier: Part I – Section D3.
[14] Dossier: Part I – Appendix 9.0 and 9.1.

Table 5: Mean values and corresponding standard deviations and ranges of protein levels (μg/g dry weight) in grain (n = 10) and leaves (n = 10) from maize Bt11 × MIR162 × 1507 × GA21

Protein	Grain (R6)	Leaves (V10/R1)
Cry1Ab	1.07[(a)] ± 0.11[(b)] [0.91–1.24][(c)]	16.49 ± 6.17 [2.58–41.93]
PAT	– [< LOD to < LOQ]	4.95 ± 0.54 [4.34–5.91]
Vip3Aa20	28.48 ± 1.37 [26.52–31.52]	74.17 ± 7.02 [50.50–110.12]
PMI	1.24 ± 0.07 [1.01–1.34]	4.79 ± 0.69 [4.1–5.71]
Cry1F	3.56 ± 0.19 [3.08–4.49]	13.32 ± 0.91 [11.06–15.11]
mEPSPS	5.15 ± 0.86 [3.52–7.09]	31.11 ± 4.01 [19.41–55.38]

LOD: values below limit of detection; LOQ: values below limit of quantification.
–: not applicable.
(a): mean.
(b): standard deviation.
(c): range.

In order to assess the changes in protein expression levels which may result from potential interactions between the events, protein levels were determined for the four-event stack and the corresponding single events in different parts of the plant. The levels of all the proteins in the four-event stack and the corresponding single events were similar in all tissues except for PAT and Cry1Ab in leaves (V10 and R1). For PAT, the difference was expected, resulting from the combination of the Bt11 and 1507 single events, both producing PAT in the four-event stack. As regards Cry1Ab, the mean Cry1Ab levels in leaves in the four-event stack were higher than in the corresponding single event (x4 for V10; x2.7 for R1). Taking into account that Cry1Ab levels in the four-event stack are similar to those of the corresponding single event in all remaining tissues, the GMO Panel does not consider the differences in the mean levels of Cry1Ab protein as significant and concludes that these data do not indicate an interaction between the events that may result in significant changes in the levels of newly expressed proteins.

3.3.1.4. Conclusion

The molecular data establish that the events stacked in maize Bt11 × MIR162 × 1507 × GA21 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. PAT showed in general the expected higher levels in the stack resulting from the combination of the Bt11 and 1507 single events. Therefore, there is no indication of an interaction that may affect the integrity of the events and the levels of the newly expressed proteins in this stack.

Based on the known biological function of the newly expressed proteins (Table 4), the only foreseen interactions at the biological level are between the two Cry proteins and between the Vip3Aa20 and the Cry proteins in susceptible insects, which are dealt with in Section 3.3.3.

3.3.2. Comparative analysis

3.3.2.1. Choice of comparator and production of material for the comparative analysis[15]

Three field trial studies for comparative analysis were assessed: one for agronomic and phenotypic characterisation, one for compositional analysis and an additional study for carotenoid composition.

Maize Bt11 × MIR162 × 1507 × GA21 was obtained by conventional crossing of the four single events. Maize events Bt11, MIR162 and GA21 were introduced in the inbred maize line NP222, while event 1507 was introduced in inbred maize line 5XH751. As documented by the pedigree, the four single events were combined in a hybrid maize with genetic background (F_1) 5XH751/NP2222. The same two inbred maize lines (5XH751 and NP2222) were crossed to produce the non-GM hybrid maize

[15] Dossier: Part I – Section D7.1.

used as comparator. On the basis of the provided pedigree, the GMO Panel considers that hybrid maize 5XH751/NP2222 is a suitable non-GM comparator.

For the analysis of agronomic and phenotypic characteristics, the four-event stack maize and its non-GM comparator maize 5XH751/NP2222 were grown in 13 locations in the USA in 2008,[16] representing the range of environmental conditions under which the four-event stack maize would typically be grown. At each location, the two test materials were grown in plots within replicated blocks (four per location) according to a randomised complete block design. Maintenance pesticide treatment was applied to both maize materials according to local requirements. No treatments of the four-event stack maize with the intended herbicides were included in the study.[17] This experimental design allows a direct comparison between the four-event stack maize and its non-GM comparator in the presence of maintenance herbicides only.

For the compositional analysis of forage and grains, a set of field trials was performed in six locations in the USA in 2008.[18] At each location, maize Bt11 × MIR162 × 1507 × GA21 and its non-GM comparator maize 5XH751/NP2222 were grown in replicated blocks (three per location) according to a randomised complete block design. Plant protection products (PPP) were applied to both materials according to local requirements. The plots with the four-event stack maize were treated with the intended herbicides (glyphosate and glufosinate-ammonium) on top of PPP.[19] This experimental design allows a direct comparison between the four-event stack maize and its non-GM comparator in the presence of intended herbicides only. Additionally, in a separate field trial study, [20] eight non-GM commercially available maize lines were grown at eight sites in the USA in 2009[21] (in a randomised complete block design with four replicates), in order to establish the range of natural variation for maize compositional parameters.

During the risk assessment, the applicant provided additional data on carotenoid composition derived from a field trial study carried out in the USA in 2016.[22] The study was performed according to the recommendations of EFSA GMO Panel (2011a). At each of eight field trial locations,[23] the following materials were grown in a randomised complete block design with four replicates: maize Bt11 × MIR162 × 1507 × GA21, the non-GM comparator maize 5XH751/NP2222 and three non-GM commercial reference varieties, all treated (sprayed) with PPP according to local requirements; and maize Bt11 × MIR162 × 1507 × GA21 treated with the intended herbicides (glyphosate and glufosinate-ammonium) in addition to PPP. A total of six non-GM reference varieties were tested in the study.[24]

3.3.2.2. Agronomic and phenotypic analysis[25,26]

Twenty-three parameters related to crop physiology, morphology, development, yield and biotic stress were measured.[27] Data collected for 10 of the 23 parameters[28] were subjected to an analysis of variance (ANOVA) across locations, in order to test for differences between the four-event stack maize and the non-GM comparator.[29] The other parameters were not analysed with formal statistical tests because they did not fulfil the assumptions of ANOVA.[30]

[16] Brookings, SD, Minnesota Lake, MN, Nothrfield, MN, Janesville, WI, New Haven, IN, Beaver Crossing, NE, El Paso, IL, Bloomington, IL, Shirley, IL, St. Joseph, IL, Mackinaw, IL, La Salle, IL, Marshall, MO.
[17] Additional information: 26/4/2016.
[18] Stanton, MN, Janesville, WI, New Haven, IN, Beaver Crossing, NE, Bloomington, IL, Marshal, MO.
[19] Application dates of intended herbicides were provided for all sites except Janesville, WI. Dossier: Part I – Appendix 15.0.
[20] Additional information: 12/3/2015.
[21] York, NB, Swanton, OH, Deerfield, MI, Richland, IO, Seymour, IL, York, NB, Kimballton, IO, Elk Horn, IO.
[22] Additional information: 4/10/2017.
[23] Atlantic, IA, Germansville, PA, Stewardson, IL, York, NE, Richland, IA, Geneva, MN, Wyoming, IL and Carlyle, IL.
[24] The six varieties were SY SINCERO, SY GENEROSO, NK PAKO, NK LUCIUS, CISKO and SY PROVIAL.
[25] Dossier: Part I – Section D7.4.
[26] Dossier: Part I – Section D7.1, Appendix 15.0, 15.2 and 15.3; additional information: 26/4/2013.
[27] Parameters analysed for agronomic and phenotypic characteristics: grain moisture, grain yield, % stalk lodging, % barren plants, ear height, % plants with dropped ears, % snapped plants, emerged plants, early emergence vigour, early growth vigour, plant population at harvest, heat units to 50% silking, heat units to 50% pollen shed, plant height, test weight, grey leaf spot, late season intactness, leaf colour rating, northern corn leaf blight, southern corn leaf blight, push test, % early root lodging and % late root lodging.
[28] Emerged plants, early emergence vigour, ear height, grain moisture, plant population at harvest, heat units to 50% silking, heat units to 50% pollen shed, plant height, test weight and grain yield.
[29] The model used for the ANOVA included the effect of genotype, location, block-within-location and genotype-by-location.
[30] % stalk lodging, % barren plants, % plants with dropped ears, % snapped plants, early growth vigour, grey leaf spot, late season intactness, leaf colour rating, northern corn leaf blight, southern corn leaf blight, push test, % early root lodging, % late root lodging. The GMO Panel also noted that some endpoints were recorded at a limited number of sites only: early emergence vigour at three sites and % early root lodging at two sites (additional information: 26/4/2013).

Statistically significant differences between the four-event stack maize and its non-GM comparator were observed for the endpoints '% emerged plants', 'heat units to 50% silking' and 'heat units to 50% pollen shed'.[31]

These significant differences are not considered relevant for human and animal health, but are further assessed for their potential environmental impact in Section 3.3.4.

3.3.2.3. Compositional analysis[32]

Grain and forage harvested from the field trials in the USA in 2008 were analysed for 65 constituents (nine in forage and 56 in grains) including the key constituents recommended by OECD (OECD, 2002). Six grain constituents were not statistically analysed.[33] The data for the remaining constituents (nine in forage[34] and 50 in grains[35]) were analysed with ANOVA, in order to test for differences between maize Bt11 × MIR162 × 1507 × GA21 and the non-GM comparator.[29] In case a significant difference was identified, the level of the parameter in the four-event stack maize was compared to the values reported in the literature and to the levels occurring in the eight commercial non-GM maize varieties grown in the USA in 2009.[20]

No significant differences in the composition of forage were observed between maize Bt11 × MIR162 × 1507 × GA21 and its non-GM comparator. Significant differences in grain composition are shown in Table 6. The levels of most amino acids were lower in maize Bt11 × MIR162 × 1507 × GA21 than in the non-GM comparator; this effect was likely linked to the difference observed in protein content. Except for β-carotene, the levels of all the significantly different endpoints fell within the range of compositional values obtained from the 2009 field trials and from the literature (OECD, 2002). The mean levels of β-carotene in the GM maize (0.031 mg/100 g dry weight (DW)) were about 50% lower than those in the non-GM comparator (0.061 mg/100 g DW); the levels also fell outside the range obtained from the 2009 field trials and were close to the lower limit described in the literature (OECD, 2002; Owens et al., 2014).

Table 6: Composition of maize grains: mean values (estimated from the 2008 field trials in the USA) of endpoints for which significant differences were found between the GM maize and the non-GM comparator

Endpoint	Maize Bt11 × MIR162 × 1507 × GA21	Non-GM comparator 5XH571/NP2222
Fat (% DW)	4.33	4.53
Crude protein (% DW)	8.88	9.40
Carbohydrates (% DW)	85.40	84.69
Iron (mg/kg DW)	19.3	20.0
Manganese (mg/kg DW)	5.65	6.41
Phosphorus (mg/kg DW)	2972	2818
Potassium (mg/kg DW)	4217	3882
Zinc (mg/kg DW)	17.7	20.2
β-carotene (mg/100 g DW)	0.031	0.061
Niacin (mg/100 g DW)	2.96	2.69
Threonine (mg/g DW)	3.09	3.27

[31] Mean values for '% emerged plants': 64 (GM maize) and 63 (non-GM comparator). Mean values for 'heat units to 50% silking': 1327 (GM maize) and 1315 (non-GM comparator). Mean values for 'heat units to 50% pollen shed': 1332 (GM maize) and 1320 (non-GM comparator).

[32] Dossier: Part I – Section D7.4, Appendix 13.0, 13.1, 13.2 and 13.3; additional information: 23/6/2014, 12/3/2015, 4/10/2017 and 5/4/2018.

[33] Levels of selenium, sodium, vitamin E, furfural and raffinose were not analysed because most of the data were below the limit of quantification. Moisture levels were not analysed as the grains were dried before the analytical measurements.

[34] Proximates (moisture, protein, fat, ash and carbohydrates), fibre fractions (acid detergent fibre (ADF) and neutral detergent fibre (NDF)) and minerals (calcium and phosphorus).

[35] Proximates (ash, carbohydrates, crude fat and protein), fibre fractions (acid detergent fibre, neutral detergent fibre and total dietary fibre (TDF)), starch, minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium and zinc), vitamins (β-carotene, thiamine, riboflavin, niacin, pyridoxine and folic acid), fatty acids (palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3)), amino acids (alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, proline, phenylalanine, proline, serine, tryptophan, tyrosine and valine) and other compounds (ferulic acid, inositol, *p*-coumaric acid, phytic acid and trypsin inhibitor).

Endpoint	Maize Bt11 × MIR162 × 1507 × GA21	Non-GM comparator 5XH571/NP2222
Serine (mg/g DW)	4.19	4.48
Glutamic acid (mg/g DW)	16.2	17.4
Proline (mg/g DW)	7.69	8.29
Glycine (mg/g DW)	3.53	3.71
Alanine (mg/g DW)	6.66	7.07
Cysteine (mg/g DW)	2.07	2.21
Methionine (mg/g DW)	1.81	1.98
Histidine (mg/g DW)	2.51	2.66
Stearic acid (% FA)	2.44	2.39
Oleic acid (% FA)	29.69	30.07
Linoleic acid (% FA)	51.83	51.63
p-Coumaric acid (mg/kg DW)	154	170
Phytic acid (% DW)	0.94	0.86

DW: dry weight; % FA: percentage of total fatty acids.

The decrease in β-carotene observed in maize Bt11 × MIR162 × 1507 × GA21 might indicate changes in the carotenoid pathway: in the synthesis of all-trans-lycopene (the precursor of both α- and β-carotene), in the synthesis of lutein (downstream of α-carotene) or in the synthesis of β-cryptoxanthin and zeaxanthin (downstream of β-carotene) (Yuan et al., 2015). In order to characterise both branches of the pathway, the GMO Panel requested analytical data on other carotenoids in the pathway in maize Bt11 × MIR162 × 1507 × GA21.

The applicant provided data on six carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein, lycopene and zeaxanthin) derived from a field trial study carried out in the USA in 2016.[22] Materials included were maize Bt11 × MIR162 × 1507 × GA21 (treated and not treated with the intended herbicides), the non-GM comparator maize 5XH751/NP2222 and six non-GM commercial varieties.

The levels for α-carotene and lycopene had more than 50% of the measurements below the limit of quantification and were consequently excluded from the statistical analysis. The data on the levels of β-carotene, β-cryptoxanthin, lutein and zeaxanthin were analysed using the tests of difference and equivalence as specified by EFSA GMO Panel (2010b, 2011a).[36]

The test of difference identified significant differences between maize Bt11 × MIR162 × 1507 × GA21 (both treated and not treated) and the non-GM comparator for all four carotenoids in grains. Zeaxanthin fell in equivalence category I, while the remaining three carotenoids fell into equivalence category III or IV. Mean estimates for the endpoints showing significant differences between maize Bt11 × MIR162 × 1507 × GA21 and the non-GM comparator and falling under category III/IV are given in Table 7.

[36] This includes, for each of the two treatments of the GM maize, the application of a difference test (between the GM maize and the non-GM comparator) and an equivalence test (between the GM maize and the set of non-GM commercial reference varieties). The results of the equivalence test are categorised into four possible outcomes: 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).

Table 7: Carotenoids from maize grains: means (for the GM maize, the non-GM comparator and the set of non-GM reference varieties) and equivalence limits (from the non-GM reference varieties) estimated from the 2016 field trials in the USA

Endpoint [(b)](mg/100 g DW)	Maize Bt11 × MIR162 × 1507 × GA21		Non-GM comparator	Non-GM reference varieties	
	Not treated[(a)]	Treated[(a)]		Mean	Equivalence limits
β-Carotene	0.0384*	0.0352*	0.0716	0.155	(0.056, 0.426)
β-Cryptoxanthin	0.0293*	0.0292*	0.0463	0.0928	(0.0347, 0.246)
Lutein	0.512*	0.500*	0.750	1.39	(0.668, 2.91)

DW: dry weight.
For the GM maize, significantly different entries are marked with an asterisk, while the outcomes of the test of equivalence are differentiated by greyscale backgrounds: light grey (equivalence category III) and dark grey (equivalence category IV).
(a): Treated: treated with glyphosate- and glufosinate-ammonium; Not treated: treated only with conventional herbicides (see Section 3.3.2.1).
(b): The GMO Panel noted that no information was provided by the applicant on the recovery of the analytical method for the carotenoids analysed. This does not affect the outcome of the comparative risk assessment in application EFSA-GMO-DE-2010-86.

3.3.2.4. Conclusion

The GMO Panel concluded that the differences in agronomic and phenotypic characteristics identified between maize Bt11 × MIR162 × 1507 × GA21 and its non-GM comparator do not require further assessment, except for '% emerged plants', 'heat units to 50% silking' and 'heat units to 50% pollen shed'. These differences are further assessed for their potential environmental impact in Section 3.3.4.

The GMO Panel assessed all the compositional differences between the GM maize and the non-GM comparator, taking into account the potential impact on plant metabolism, the variability reported in the literature and, for carotenoid composition, the natural variability observed for the set of non-GM reference varieties. The GMO Panel concluded that no differences require further assessment for food/feed safety except those observed for β-carotene, β-cryptoxanthin and lutein (Section 3.3.3).

3.3.3. Food and feed safety assessment

3.3.3.1. Effect of processing[37]

Maize Bt11 × MIR162 × 1507 × GA21 will undergo existing production processes used for conventional maize. No novel production process is envisaged. Therefore, processing of maize Bt11 × MIR162 × 1507 × GA21 into food and feed products is not expected to result in products being different from those derived from non-GM varieties.

3.3.3.2. Toxicology

Toxicological assessment of newly expressed proteins

Six proteins are newly expressed in the four-event stack maize (Section 3.3.1.3). The GMO Panel previously assessed these proteins individually in the context of the single events, and no safety concern was identified.

The three enzymatic proteins (PAT, PMI and mEPSPS) act on unrelated substrates and are not expected to interact. The three insecticidal proteins (Cry1Ab, Vip3Aa20 and Cry1F) act through cellular receptors found in target insect species. It is reported that the gastrointestinal tract of mammals, including humans, lacks receptors with specific high affinity to Cry proteins (Hammond et al., 2013; Koch et al., 2015).

On the basis of the known biological function of the newly expressed proteins (Table 4), there is currently no expectation for possible interactions relevant to the food and feed safety assessment of the four-event stack maize Bt11 × MIR162 × 1507 × GA21.

The GMO Panel concludes that based on current knowledge, there are no safety concerns to human and animal health related to the newly expressed proteins Cry1Ab, PAT, Vip3Aa20, PMI, Cry1F and mEPSPS in the four-event stack maize Bt11 × MIR162 × 1507 × GA21.

[37] Dossier: Part I – Section D7.6.

Toxicological assessment of components other than newly expressed proteins

The compositional differences observed in the four-event stack maize with respect to its non-GM comparator did not require toxicological assessment (see Section 3.3.2.3).

3.3.3.3. Animal studies with the food/feed derived from GM plants

No animal studies with food/feed derived from maize Bt11 × MIR162 × 1507 × GA21 were provided by the applicant. No compositional modifications relevant for safety or nutrition are expected in food and feed derived from the four-event stack maize (Sections 3.3.2 and 3.3.3.5), and there was no indication of interactions relevant for food/feed safety (Sections 3.3.1 and 3.3.2). Therefore, animal feeding studies are not considered necessary (EFSA, 2006; EFSA GMO Panel, 2011a).

3.3.3.4. Allergenicity

For the allergenicity assessment, a weight-of-evidence approach was followed, taking into account all of the information obtained on the newly expressed proteins, since no single piece of information or experimental method yields sufficient evidence to predict allergenicity (EFSA, 2006; Codex Alimentarius, 2009). In addition, when known functional aspects of the newly expressed protein or structural similarity to known adjuvants may indicate an adjuvant activity, the possible role of these proteins as adjuvants is considered. When newly expressed proteins with a potential adjuvant activity are expressed together, possible interactions increasing adjuvanticity and impacting the allergenicity of the GM crop are assessed.

Assessment of allergenicity of the newly expressed proteins[38]

The GMO Panel previously evaluated the safety of the Cry1Ab, Cry1F, Vip3Aa20, PAT, mEPSPS and PMI proteins individually, and no concerns on allergenicity were identified in the context of the applications assessed (see EFSA scientific opinions listed in Table 2). No new information on allergenicity of these proteins that might change the previous conclusions of the GMO Panel has become available. Based on current knowledge, and as none of the newly expressed proteins showed allergenicity, no reasons for concerns regarding the simultaneous presence of these newly expressed proteins in this four-event stack maize affecting allergenicity were identified.

For adjuvanticity, proteins derived from *Bacillus thuringiensis* (Bt proteins) have been suggested to possess adjuvant activity, based on animal studies on Cry1Ac when applied at relatively high doses (e.g. Vázquez et al., 1999). The Panel previously evaluated the safety of the Cry1Ab, Cry1F and Vip3Aa20 proteins and no concerns on adjuvanticity in the context of the applications assessed were identified (see EFSA scientific opinions listed in Table 2). The levels of Bt proteins in the four-event stack maize are similar to those in the respective single maize events (see Table 5). From the limited experimental evidence available, the GMO Panel did not find indications that the presence of the Bt proteins at the levels expressed in the four-event stack maize might act as adjuvants with the potential to enhance a specific immunoglobulin E (IgE) response and to favour the development of an allergic reaction.

Assessment of allergenicity of GM plant products[39]

The GMO Panel regularly reviews the available publications on food allergy to maize. However, to date, maize has not been considered to be a common allergenic food[40] (OECD, 2002). Therefore, the GMO Panel did not request experimental data to analyse the allergen repertoire of GM maize.

In the context of this application and considering the data from the molecular characterisation, the compositional analysis and the assessment of the newly expressed proteins (see Sections 3.3.1, 3.3.2.3 and 3.3.3.2), the GMO Panel identified no indications of a potentially increased allergenicity of food and feed derived from the four-event stack maize with respect to that derived from its non-GM comparator.

[38] Dossier: Part I – Section D7.9; additional information: 30/7/2015; 17/12/2015 (spontaneous submission), 4/4/2016 and 26/4/2013.
[39] Dossier: Part I – Section D7.9.
[40] Directive 2007/68/EC of the European Parliament and of the Council of 27 November 2007 amending Annex IIIa to Directive 2000/13/EC of the European Parliament and of the Council as regards certain food ingredients. OJ L 310, 27.11.2007, p. 11–14.

3.3.3.5. Nutritional assessment of GM food/feed[41]

The intended traits of maize Bt11 × MIR162 × 1507 × GA21 are herbicide tolerance and insect resistance, with no intention to alter the nutritional parameters. However, the levels of β-carotene, β-cryptoxanthin and lutein measured in the field trials in 2016 in both treated and non-treated GM maize were significantly different from its non-GM comparator and showed a lack of equivalence with the reference varieties (Section 3.3.2.3). The biological role of these compounds, their levels in maize and maize-derived products and the magnitude and direction of the observed changes were considered during the nutritional assessment.

Human nutrition

Levels of β-carotene and β-cryptoxanthin in both treated and non-treated maize Bt11 × MIR162 × 1507 × GA21 analysed in the field trials carried out in 2016 were lower than those reported for the non-GM comparator: about 50% lower for β-carotene and about 40% lower for β-cryptoxanthin (Section 3.3.2.3). Both carotenoids are precursors of vitamin A and are found in plant-derived foods; together with preformed vitamin A (mainly retinol and retinyl esters) present in foods of animal origin, they contribute to the total dietary intake of vitamin A. Milk, meat, vegetables and derived products are the main sources of vitamin A in the diet (EFSA NDA Panel, 2015). Cereals and cereal-based products contribute much less to the total intake of vitamin A, with contributions in the adult population ranging between 2.7% and 6.5% of the total (average contribution = 4.1%), with the maximum contribution (10%) estimated in adolescents (EFSA NDA Panel, 2015). Similar contribution by cereals and cereal-based products is observed in the young population (toddlers, 1–3 years old) with up to 7% of total vitamin A intake. In this age class, there are specifically manufactured foods ('Food products for young population') that are supplemented with vitamin A in order to reach the regulated levels;[42,43] in most cases, they are supplemented with preformed vitamin A (retinol) rather than with β-carotene. The contribution of these foods to the total vitamin A intake can be up to 16% (EFSA NDA Panel, 2015).

Levels of lutein in both treated and non-treated maize Bt11 × MIR162 × 1507 × GA21 were about 35% lower than those reported for the non-GM comparator. Unlike for vitamin A, no recommended dietary intake levels for lutein have been established, as the evidence provided is insufficient to establish a cause–effect relationship between consumption of lutein and maintenance of normal vision (EFSA NDA Panel, 2010, 2012). Lutein is together with zeaxanthin the dominant carotenoid in maize kernels, although their levels are much lower than those identified in green leafy vegetables such as spinach, kale and parsley (Humphries and Khachik, 2003; Abdel-Aal et al., 2013; Eisenhauer et al., 2017). Even though in most current nutrition databases the content of lutein is given together with zeaxanthin, it is known that, in general, zeaxanthin and not lutein is the predominant carotenoid in maize and maize-based products, while the opposite is observed in green leafy vegetables (Humphries and Khachik, 2003; Perry et al., 2009).

Although a significantly lower level of β-carotene and β-cryptoxanthin was observed in maize Bt11 × MIR162 × 1507 × GA21 compared to its non-GM comparator, the current available consumption data indicate a minor contribution of maize and maize-derived products to total vitamin A intake. Based on this, the GMO Panel concludes that foods derived from maize Bt11 × MIR162 × 1507 × GA21 have the same nutritional impact as regards the total vitamin A intake as those derived from its non-GM comparator and the non-GM commercial reference varieties. Similarly, due to the current contribution of maize and maize-derived products to the total dietary intake of lutein and considering the unproven beneficial effects of lutein on health, the GMO Panel concludes that no adverse effects on human nutrition from the consumption of maize Bt11 × MIR162 × 1507 × GA21 can be expected.

Animal nutrition

Considering that the significance of β-carotene/β-cryptoxanthin in diets for animals is negligible because these are supplemented with vitamin A, the GMO Panel is of the view that the observed lower level of β-carotene in maize Bt11 × MIR162 × 1507 × GA21 would not affect its total dietary intake in the European animal population. Other carotenoids, such as lutein and zeaxanthin, are used as

[41] Dossier: Part I – Section D7.10.
[42] COMMISSION DIRECTIVE 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC.
[43] COMMISSION DIRECTIVE 2006/125/EC of 5 December 2006 on processed cereal-based foods and baby foods for infants and young children.

colourants in poultry for yolk and skin colouring (EFSA, 2009c), but are not recognised as essential nutrients for animals.

3.3.3.6. Conclusion

The GMO Panel considers that the newly expressed proteins in the four-event stack maize do not raise safety concerns for human and animal health and that, on the basis of the biological properties of the newly expressed proteins, no interactions relevant to food and feed are expected between these proteins. The GMO Panel did not identify safety concerns regarding allergenicity or adjuvanticity with the presence of newly expressed proteins in this four-event stack maize, or regarding the overall allergenicity of the four-event stack maize. The GMO Panel concludes that foods and feeds derived from maize Bt11 × MIR162 × 1507 × GA21 are expected to have the same nutritional impact as those derived from non-GM commercial maize varieties.

3.3.4. Environmental risk assessment[44]

Considering the scope of application EFSA-GMO-DE-2010-86, which excludes cultivation, the ERA of maize Bt11 × MIR162 × 1507 × GA21 mainly takes into account: (1) exposure of microorganisms to recombinant DNA in the gastrointestinal tract of animal fed GM material and of microorganisms present in environments exposed to faecal material of these animals (manure and faeces); and (2) the accidental release into the environment of viable maize Bt11 × MIR162 × 1507 × GA21 grains during transportation and processing (EFSA GMO Panel, 2010a).

3.3.4.1. Persistence and invasiveness of the GM plant[45]

Maize is highly domesticated, not winter hardy in colder regions of Europe and generally unable to survive in the environment without appropriate management. Occasional feral GM maize plants may occur outside cultivation areas in the EU (e.g. Pascher, 2016), but survival is limited mainly by a combination of low competitiveness, absence of a dormancy phase and susceptibility to plant pathogens, herbivores and cold climate conditions (OECD, 2003). Field observations indicate that maize grains may survive and overwinter in some EU regions, resulting in volunteers in subsequent crops (e.g. Gruber et al., 2008; Palaudelmàs et al., 2009; Pascher, 2016). However, maize volunteers have been shown to grow weakly and flower asynchronously with the maize crop (Palaudelmàs et al., 2009). Thus, the establishment and survival of feral and volunteer maize in the EU is currently limited and transient.

It is unlikely that the intended traits of maize Bt11 × MIR162 × 1507 × GA21 will provide a selective advantage to maize plants, except when they are exposed to glyphosate- and/or glufosinate-ammonium-containing herbicides or infested by insect pests that are susceptible to the Cry1Ab, Cry1F and/or Vip3Aa20 proteins.

The GMO Panel considers that the fitness advantage provided by the intended traits, and the observed differences in emerged plants, heat units to 50% silking and 50% pollen shed (see Section 3.3.2.2) will not allow the GM plant to overcome other biological and abiotic factors (described above) limiting plant's persistence and invasiveness. Therefore, the presence of the intended traits and other observed differences will not affect the persistence and invasiveness of the GM plant.

In conclusion, the GMO Panel considers it very unlikely that maize Bt11 × MIR162 × 1507 × GA21 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 Bt11 × MIR162 × 1507 × GA21 grains.

3.3.4.2. Potential for gene transfer[46]

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

Plant-to-microorganism gene transfer

The probability and potential adverse effects of HGT of the recombinant DNA have been assessed in the previous GMO Panel Scientific Opinions on the single events (see Table 2). No concern as a result of an unlikely, but theoretically possible, HGT of the recombinant genes to bacteria in the gut of

[44] Dossier: Part I – Section D9.
[45] Dossier: Part I – Section D9.1 and D9.2.
[46] Dossier: Part I – Section D9.3.

domesticated animals and humans fed GM material or other receiving environments was identified. 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 four-event stack maize to bacteria does not raise any environmental safety concern.

Plant-to plant-gene transfer

The potential for occasional feral maize Bt11 × MIR162 × 1507 × GA21 plants originating from grain import spills to transfer recombinant DNA to sexually compatible plants and the environmental consequences of this transfer were considered.

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

Maize is an annual predominantly cross-pollinating crop. Cross-fertilisation occurs mainly by wind (OECD, 2003). Vertical gene transfer from maize is limited to *Zea* species. Wild relatives of maize outside cultivation are not known/reported in Europe (Eastham and Sweet, 2002; OECD, 2003; EFSA, 2016; Trtikova et al., 2017). Therefore, potential vertical gene transfer is restricted to maize and weedy *Zea* species, such as teosintes and/or maize–teosinte hybrids, occurring in cultivated areas (EFSA, 2016; Trtikova et al., 2017).

The potential of spilled maize grains to establish, grow and produce pollen is extremely low and transient (see Section 3.3.4.1). 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 3.3.4.1.

3.3.4.3. Interactions of the GM plant with target organisms[47]

Taking the scope of application EFSA-GMO-DE-2010-86 into account, potential interactions of occasional feral maize Bt11 × MIR162 × 1507 × GA21 plants with the target organism arising from grain import spills are not considered a relevant issue.

3.3.4.4. Interactions of the GM plant with non-target organisms[48]

Given that environmental exposure of non-target organisms to spilled GM grains or occasional feral GM maize plants arising from spilled maize Bt11 × MIR162 × 1507 × GA21 grains is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions of maize Bt11 × MIR162 × 1507 × GA21 with non-target organisms are not considered to raise any environmental safety concern. Interactions that may occur between the Cry and Vip proteins (as mentioned in Section 3.3.1.1) will not alter this conclusion.

3.3.4.5. Interactions with the abiotic environment and biogeochemical cycles[49]

Given that environmental exposure to spilled grains or occasional feral maize Bt11 × MIR162 × 1507 × GA21 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 to raise any environmental safety concern.

3.3.4.6. Conclusion of the environmental risk assessment

The GMO Panel concludes that it is unlikely that maize Bt11 × MIR162 × 1507 × GA21 would differ from conventional maize varieties in its ability to persist under European environmental conditions. Considering the scope of application EFSA-GMO-DE-2010-86, interactions of occasional feral maize Bt11 × MIR162 × 1507 × GA21 plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from maize Bt11 × MIR162 × 1507 × GA21 to

[47] Dossier: Part I – Section D9.4.
[48] Dossier: Part I – Section D9.5.
[49] Dossier: Part I – Section D9.8.

bacteria does not indicate a safety concern. Therefore, considering the combined traits and their interactions, the outcome of the comparative analysis and the routes and levels of exposure, the GMO Panel concludes that maize Bt11 × MIR162 × 1507 × GA21 would not raise safety concerns in the event of accidental release of viable GM maize grains into the environment.

3.3.5. Conclusion on maize Bt11 × MIR162 × 1507 × GA21

No new data on the single maize events Bt11, MIR162, 1507 and GA21 leading to a modification of the original conclusions on their safety were identified.

Based on the molecular characterisation and on the comparative analysis of agronomic, phenotypic and compositional characteristics, the combination of maize events Bt11, MIR162, 1507 and GA21 in the four-event stack maize did not give rise to food and feed safety issues. Based on the nutritional assessment of the compositional characteristics of maize Bt11 × MIR162 × 1507 × GA21, foods and feeds derived from the GM maize are expected to have the same nutritional impact as those derived from non-GM maize varieties.

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.

Based on the biological functions of the newly expressed proteins, no indications of interactions between the events were identified that would raise a safety issue. Comparison of the levels of the newly expressed proteins between the four-event stack and each of the single events did not reveal an interaction at protein expression level.

Considering the combined traits and their interactions, the outcome of the comparative analysis and routes and levels of exposure, the GMO Panel concludes that maize Bt11 × MIR162 × 1507 × GA21 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 was retrieved in a literature search covering the period since the time of validity of the application.[50]

The GMO Panel concludes that the four-event stack maize is nutritionally equivalent to and as safe as its non-GM comparator in the context of the scope of this application.

3.4. Risk assessment of the subcombinations

3.4.1. Subcombinations previously assessed

In previous assessments, the GMO Panel identified no safety concerns for the seven subcombinations of maize Bt11 × MIR162 × 1507 × GA21 not included in the scope of application EFSA-GMO-DE-2010-86 (Table 2). No new scientific information relevant to the risk assessment of these subcombinations became available since the validation of the application. Consequently, the GMO Panel considers that its previous conclusions on these subcombinations remain valid.

3.4.2. Subcombinations not previously assessed

The three subcombinations of maize Bt11 × MIR162 × 1507 × GA21 included in the scope of this application have not been previously assessed: the three-event stacks MIR162 × 1507 × GA21 and Bt11 × MIR162 × 1507 and the two-event stack MIR162 × 1507 (Table 1). No experimental data were provided for these maize stacks. The strategy followed for the assessment of subcombinations for which no specific data have been submitted, and which have not been previously assessed, has been described by the GMO Panel.[51] 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.

A literature search revealed no scientific information relevant to the risk assessment of the three subcombinations that became available since the validation of application EFSA-GMO-DE-2010-86.[50]

3.4.2.1. 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

[50] Additional information: 3/11/2016.
[51] 115th GMO Panel meeting (Annex 1 of the minutes: http://www.efsa.europa.eu/sites/default/files/event/170517-m.pdf).

stack Bt11 × MIR162 × 1507 × GA21 (Section 3.3.1) and in the previously assessed subcombinations (Table 2). The GMO Panel finds no reasons to expect loss of integrity of the events in the subcombinations MIR162 × 1507 × GA21, Bt11 × MIR162 × 1507 and MIR162 × 1507.

3.4.2.2. Expression of the events

The GMO Panel assessed whether the combination of any 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 interaction between the events present in the three subcombinations. Based on current knowledge of the molecular elements introduced, there is no reason to expect interactions that would affect the levels of the newly expressed proteins in the 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, except for the differences observed for Cry1Ab in leaves and for the expected differences in PAT levels resulting from the combination of the Bt11 and 1507 events both producing PAT protein (Section 3.3.1.3 and Appendix A). Therefore, there is no indication of an interaction manifesting at protein expression level. In addition, protein expression data from the two-event stacks Bt11 × GA21 (EFSA GMO Panel, 2009a) and Bt11 × MIR162 (EFSA GMO Panel, 2017c) and the three-event stack Bt11 × 1507 × GA21 (EFSA GMO Panel, 2017d) were either similar to those observed in each of the single maize events or, for PAT in maize Bt11 × 1507 × GA21, showed the expected higher levels resulting from the combination of the Bt11 and 1507 events, both producing PAT protein. This confirms that interactions affecting expression levels of the newly expressed proteins are not expected in the subcombinations MIR162 × 1507 × GA21, Bt11 × MIR162 × 1507 and MIR162 × 1507 included in the scope of this application.

3.4.2.3. Potential interactions between the events

The GMO Panel assessed the potential interactions between events due to their combination in maize MIR162 × 1507 × GA21, maize Bt11 × MIR162 × 1507 and maize MIR162 × 1507, taking into consideration intended traits and identified potential 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/feed or environmental safety between these proteins in any of the three subcombinations, taking into account the scope of this application.

The GMO Panel took into account the intended and potential unintended effects considered in the assessment of the four single events, of the previously assessed subcombinations (Table 2) and of the four-event stack maize Bt11 × MIR162 × 1507 × GA21. It was concluded that none of these effects would raise safety concerns when combined in any of the three maize subcombinations. Therefore, the GMO Panel is of the opinion that no additional data are needed to complete the assessment of the three subcombinations.

3.4.3. Conclusion

For the three subcombinations included in the scope of application EFSA-GMO-DE-2010-86, no experimental data have been provided. The GMO Panel assessed the possibility of interactions between the events and concluded that these combinations do not raise safety concerns. The three subcombinations are therefore expected to be as safe as the single maize events, the previously assessed subcombinations (Table 2) and the four-event stack maize Bt11 × MIR162 × 1507 × GA21.

3.5. Post-market monitoring

3.5.1. Post-market monitoring of GM food/feed[52]

No compositional changes relevant for food/feed safety or nutrition were identified in maize Bt11 × MIR162 × 1507 × GA21 when compared with the non-GM comparator. The GMO Panel considers maize Bt11 × MIR162 × 1507 × GA21 to be as safe as the non-GM comparator. The three subcombinations not previously assessed and included in the scope of application EFSA-GMO-DE-2010-86 are expected to be as safe as the single maize events, the four-event stack maize

[52] Dossier: Part I – Section D7.11.

Bt11 × MIR162 × 1507 × GA21 and the previously assessed subcombinations. Therefore, the GMO Panel considers that post-market monitoring (EFSA, 2006) of food/feed derived from maize Bt11 × MIR162 × 1507 × GA21 and its three subcombinations is not necessary.

3.5.2. Post-market environmental monitoring[53]

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 did not identify potential adverse environmental effects from maize Bt11 × MIR162 × 1507 × GA21 (Section 3.3.4), no case-specific monitoring is required.

The PMEM plan proposed by the applicant for maize Bt11 × MIR162 × 1507 × GA21 includes (1) the description of a monitoring approach involving operators (federations involved in import and processing), reporting to 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 the information recorded by the various operators; and (3) 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 maize Bt11 × MIR162 × 1507 × GA21. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

4. Overall conclusions and recommendations

No new information on the single maize events Bt11, MIR162, 1507 and GA21 that would lead to a modification of the original conclusions on their safety were identified.

The combination of events Bt11, MIR162, 1507 and GA21 in the four-event stack maize did not give rise to issues relating to molecular, agronomic/phenotypic and compositional characteristics regarding food and feed safety. The newly expressed proteins in the four-event stack maize did not raise concerns for human and animal health. Foods and feeds from maize Bt11 × MIR162 × 1507 × GA21 are expected to have the same nutritional impact as those derived from the non-GM comparator.

The GMO Panel concluded that there is a very low likelihood of environmental effects resulting from the accidental release of viable grains from maize Bt11 × MIR162 × 1507 × GA21 into the environment.

The GMO Panel concludes that maize Bt11 × MIR162 × 1507 × GA21 is nutritionally equivalent to and as safe as its non-GM comparator in the context of the scope of this application.

The three subcombinations included in the scope of this application were not previously assessed. For these subcombinations, for which no experimental data were provided, the GMO Panel assessed the possibility of interactions between the events and concluded that these combinations would not raise safety concerns. The three subcombinations not previously assessed are expected to be as safe as the single maize events, the previously assessed subcombinations and the four-event stack maize Bt11 × MIR162 × 1507 × GA21.

Given that no safety concerns were identified for food and feed derived from maize Bt11 × MIR162 × 1507 × GA21 and its three subcombinations, the GMO Panel considers that post-market monitoring of these products is not necessary. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of maize Bt11 × MIR162 × 1507 × GA21 and its three subcombinations.

A minority opinion expressed by a GMO Panel member is presented in Appendix B.

Documentation as provided to EFSA

1) Letter from the Competent Authority of Germany, dated 10 August 2010, concerning a request for placing on the market of maize Bt11 × MIR162 × 1507 × GA21 in accordance with Regulation (EC) No 1829/2003.

[53] Dossier: Part I – Section D11 and Appendix 27.

2) Acknowledgement letter, dated 27 September 2010, from EFSA to the Competent Authority of Germany.
3) Letter from EFSA to applicant, dated 11 October 2010, requesting additional information under completeness check.
4) Letter from applicant to EFSA, received on 26 January 2011, providing additional information under completeness check.
5) Letter from EFSA to applicant, dated 4 March 2011, requesting additional information under completeness check.
6) Letter from applicant to EFSA, received on 25 March 2011, providing additional information under completeness check.
7) Letter from applicant to EFSA, received on 04 July 2011, extending the timeline for responses.
8) Letter from applicant to EFSA, received on 12 December 2011, extending the timeline for responses.
9) Letter from applicant to EFSA, received on 21 December 2011, providing additional information under completeness check.
10) Letter from EFSA to applicant, dated 25 January 2012, requesting additional information under completeness check.
11) Letter from applicant to EFSA, received on 15 March 2012, clarifying the scope of the application.
12) Letter from applicant to EFSA, received on 02 April 2012, providing additional information under completeness check. Letter from EFSA to applicant, dated 6 June 2012, confirming the scope of the application.
13) Letter from EFSA to applicant, dated 14 June 2012, delivering the 'Statement of Validity' for application EFSA-GMO-DE-2010-86, maize Bt11 × MIR162 × 1507 × GA21 submitted by Syngenta under Regulation (EC) No 1829/2003.
14) Letter from EFSA to applicant dated 31 October 2012 requesting additional information and stopping the clock on behalf of the EURL-GMFF.
15) Letter from EFSA to applicant dated 7 December 2012 requesting additional information and maintaining the clock stopped.
16) Letter from EFSA to applicant dated 13 December 2012 re-starting the clock on behalf of the EURL-GMFF and maintaining the clock stopped by EFSA.
17) Letter from EFSA to applicant, dated 5 February 2013, requesting additional information.
18) Letter from applicant to EFSA, received on 26 April 2013, providing additional information.
19) Letter from EFSA to applicant, dated 27 September 2013, requesting additional information and maintaining the clock stopped.
20) Letter from applicant to EFSA, received on 04 November 2013, extending the timeline for responses.
21) Letter from applicant to EFSA, received on 18 February 2014, redefining the scope of the application.
22) Letter from EFSA to applicant dated 4 June 2014 requesting additional information and maintaining the clock stopped.
23) Letter from applicant to EFSA, received on 10 June 2014, extending the timeline for responses.
24) Letter from applicant to EFSA, received on 16 June 2014, providing additional information.
25) Letter from applicant to EFSA, received on 23 June 2014, providing additional information.
26) Letter from applicant to EFSA, received on 28 July 2014, spontaneously providing additional information.
27) Letter from EFSA to applicant, dated 16 September 2014, requesting additional information and maintaining the clock stopped.
28) Letter from applicant to EFSA, received on 30 September 2014, providing additional information.
29) Letter from EFSA to applicant, dated 3 October 2014, requesting additional information and maintaining the clock stopped.
30) Letter from EFSA to applicant, dated 10 November 2014, requesting additional information and maintaining the clock stopped.
31) Letter from applicant to EFSA, received on 21 November 2014, providing additional information.

32) Letter from applicant to EFSA, received on 28 November 2014, providing additional information.
33) Letter from applicant to EFSA, dated 23 December 2014, extending the timeline for responses.
34) Letter from applicant to EFSA, received on 12 March 2015, providing additional information.
35) Letter from applicant to EFSA received on 27 May 2015 asking for clarifications on the additional information requested by EFSA on 10 November 2015.
36) Letter from EFSA to applicant dated 17 June 2015 providing clarifications requested on 27 May 2015.
37) Letter from applicant to EFSA, received on 21 July 2015, providing sequencing information spontaneously.
38) Letter from applicant to EFSA, received on 30 July 2015 (resubmitted on 10 August 2015), providing additional information.
39) Letter from applicant to EFSA, received on 9 September 2015, spontaneously providing additional information.
40) Letter from EFSA to applicant, dated 22 September 2015, requesting additional information and maintaining the clock stopped.
41) Letter from applicant to EFSA, received on 24 September 2015, providing additional information.
42) Letter from EFSA to applicant, dated 23 October 2015, restarting the clock.
43) Letter from applicant to EFSA, received on 8 December 2015, redefining the scope of the application.
44) Letter from applicant to EFSA, received on 17 December 2015, spontaneously providing additional information.
45) Letter from EFSA to applicant, dated 23 December 2015, requesting additional information and stopping the clock.
46) Letter from applicant to EFSA, received on 4 April 2016, redefining the scope of the application.
47) Letter from applicant to EFSA, received on 4 April 2016, providing additional information.
48) E-mail from EFSA to applicant, dated 11 April 2016, restarting the clock from 4 April 2016.
49) Letter from EFSA to applicant, dated 4 August 2016, requesting additional information and stopping the clock.
50) Letter from applicant to EFSA, received on 3 November 2016, providing additional information.
51) E-mail from EFSA to applicant, dated 4 November 2016, restarting the clock from 3 November 2016
52) Letter from applicant to EFSA, received on 25 November 2016, spontaneously providing additional information.
53) Letter from EFSA to applicant, dated 1 February 2017, requesting additional information and stopping the clock.
54) Letter from applicant to EFSA, dated 16 March 2017, extending the timeline for responses.
55) Letter from EFSA to applicant, dated 18 May 2017, requesting additional information and maintaining the clock stopped.
56) Letter from applicant to EFSA, received on 12 June 2017, providing additional information.
57) Letter from applicant to EFSA, dated 3 July 2017, extending the timeline for responses.
58) Letter from applicant to EFSA, received on 4 October 2017, spontaneously providing additional information.
59) Letter from applicant to EFSA, received on 14 December 2017, providing additional information.
60) E-mail from EFSA to applicant, dated 18 December 2017, restarting the clock from 14 December 2017.
61) Letter from EFSA to applicant, dated 20 December 2017, requesting additional information and stopping the clock.
62) Letter from EFSA to applicant, dated 18 January 2018, requesting additional information and maintaining the clock stopped.
63) Letter from applicant to EFSA, dated 2 February 2018, extending the timeline for responses.

64) Letter from EFSA to applicant, dated 16 February 2018, requesting additional information and maintaining the clock stopped.
65) Letter from applicant to EFSA, received on 5 March 2018, providing additional information.
66) Letter from applicant to EFSA, received on 5 April 2018, providing additional information.
67) Letter from applicant to EFSA, received on 30 April 2018, providing additional information.
68) E-mail from EFSA to applicant, dated 30 April 2018, restarting the clock.

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Abbreviations

ADF	acid detergent fibre
ANOVA	analysis of variance
bp	base pair
Cry	Crystal protein
DW	dry weight
ERA	environmental risk assessment
ELISA	enzyme-linked immunosorbent assay
FA	fatty acid
GM	genetically modified
GMO	genetically modified organism
GMO Panel	EFSA Panel on Genetically Modified Organisms
HGT	horizontal gene transfer
IgE	immunoglobulin E
mEPSPS	mutated 5-enolpyruvyl-shikimate-3-phosphate synthase
MO	minority opinion
NEP	newly expressed proteins
OECD	Organisation for Economic Co-operation and Development
ORF	open-reading frame
PAT	phosphinothricin acetyltransferase
PMEM	post-market environmental monitoring
PMI	phosphomannose isomerase
PPP	Plant protection products
TDF	total dietary fibre
UTR	untranslated region

Appendix A – Protein expression data

Means, standard deviation and ranges of protein levels (μg/g dry weight) from maize Bt11 × MIR162 × 1507 × GA21, Bt11, MIR162, 1507 and GA21 from field trials performed in USA in 2008[a]

	Bt11 × MIR162 × 1507 × GA21	Bt11	MIR162	1507	GA21
Cry1Ab					
Leaf (V10)	15.87[b] ± 10.53[c] (3.25–41.50)[d]	3.98 ± 10.53 (1.76–8.93)			
Leaf (R1)	16.49 ± 6.17 (2.58–41.93)	5.89 ± 6.17 (2.70–15)			
Root (R1)	18.66 ± 3.8 (13.07–29.83)	13.60 ± 3.8 (9.28–17.35)			
Pollen (R1)	– < LOQ[f]	– < LOQ			
Grain (R6)	1.07 ± 0.11 (0.91–1.24)	0.91 ± 0.11 (0.65–1.15)			
Whole Plant(R1)	3.84 ± 1.22 (1.51–10.28)	2.24 ± 1.22 (1.22–5.14)			
PMI					
Leaf (V10)	4.79 ± 0.69 (4.10–5.71)		4.25 ± 0.69 (3.55–5.42)		
Leaf (R1)	3.81 ± 0.19 (2.41–5.95)		4.29 ± 0.19 (2.80–5.45)		
Root (R1)	2.43 ± 0.34 (1.31–3.71)		2.54 ± 0.34 (1.62–3.21)		
Pollen (R1)	2.89 ± 0.41 (2.59–3.33)		3.31 ± 0.41 (3–3.71)		
Grain (R6)	1.24 ± 0.07 (1.01–1.34)		1.29 ± 0.07 (1.05–1.56)		
Whole Plant(R1)	1.97 ± 0.09 (1.60–2.51)		2.19 ± 0.09 (1.8–2.66)		
Vip3Aa20					
Leaf (V10)	74.05 ± 9.01 (55.3–100.13)		59.38 ± 9.01 (43.61–69.14)		
Leaf (R1)	74.17 ± 7.02 (50.50–110.12)		80.44 ± 7.02 (65.54–94.67)		
Root (R1)	30.27 ± 2.5 (25.43–34.31)		33.39 ± 2.5 (25.41–39.89)		
Pollen (R1)	41.15 ± 6.17 (36.86–44.42)		47.95 ± 6.17 (41.51–59.91)		
Grain (R6)	28.48 ± 1.37 (26.52–31.52)		29.02 ± 1.37 (25.92–32.64)		
Whole Plant(R1)	23.43 ± 1.61 (20.75–25.62)		30 ± 1.61 (25.40–33.92)		
Cry1F					
Leaf (V10)	13.32 ± 0.91 (11.06–15.11)			13.83 ± 0.91 (11.83–21.08)	
Leaf (R1)	11.67 ± 4.11 (1.79–19.30)			17.17 ± 4.11 (15.13–19.55)	
Root (R1)	5.52 ± 0.61 (4.4–7.71)			6.29 ± 0.61 (4.63–8.85)	
Pollen (R1)	21.79 ± 1.41 (20.3–23.51)			22.69 ± 1.41 (19.96–26.85)	

	Bt11 × MIR162 × 1507 × GA21	Bt11	MIR162	1507	GA21
Grain (R6)	3.56 ± 0.19 (3.08–4.49)			3.35 ± 0.19 (2.75–4.28)	
Whole Plant(R1)	4.84 ± 0.93 (3.38–7.69)			5.88 ± 0.93 (3.72–7.82)	
mEPSPS					
Leaf (V10)	31.11 ± 4.01 (19.41–55.38)				31.03 ± 4.01 (22.85–42.09)
Leaf (R1)	12.74 ± 0.77 (8.24–18.35)				21.30 ± 0.77 (16.83–28.26)
Root (R1)	10.74 ± 2.03 (5.7–21.93)				14.08 ± 2.03 (8.36–23.09)
Pollen (R1)	224.06 ± 24.35 (191.48–266.76)				179.84 ± 24.35 (161.96–208.75)
Grain (R6)	5.15 ± 0.86 (3.52–7.09)				4.73 ± 0.86 (2.58–6.56)
Whole Plant(R1)	9.78 ± 1.32 (7.47–12.14)				10.75 ± 1.32 (7.56–12.55)
PAT[(e)]					
Leaf (V10)	4.95 ± 0.54 (4.34–5.91)	0.31 ± 0.09 (0.26–0.56)		4.72 ± 1.08 (3.30–7.18)	
Leaf (R1)	4.72 ± 1.92 (1.72–6.85)	0.58 ± 0.07 (0.42–0.68)		6.26 ± 0.74 (4.94–7.20)	
Root (R1)	0.72 ± 0.19 (0.33–0.95)	0.73 ± 0.14 (0.51–0.98)		0.35 ± 0.07 (0.23–0.47)	
Pollen (R1)	– < LOD[(f)]	– < LOD		– < LOD	
Grain (R6)	– < LOD–< LOQ	– < LOD–< LOQ		– < LOD	
Whole Plant(R1)	1.02 ± 0.38 (0.51–1.67)	0.58 ± 0.12 (0.41–0.75)		1.09 ± 0.33 (0.64–1.7)	

(a): Number of samples is n = 10 except for: pollen (n = 5); PMI and Vip3Aa20 in leaves V10 (n = 8 for MIR162); mEPSPS in leaves V10 (n = 9 for GA21).
(b): Mean.
(c): Standard deviation.
(d): Range.
(e): The standard deviation (SD) values reported for PAT levels in Bt11, 1507 and Bt11 × MIR162 × 1507 × GA21 were determined with descriptive statistics. The SD values for Cry1Ab, PMI, Vip3Aa20, Cry1F and mEPSPS levels were determined with analysis of variance.
(f): LOD: limit of detection; LOQ: limit of quantification; –: not applicable.

Appendix B – Minority opinion

Application EFSA-GMO-DE-2010-86 (Bt11 × MIR162 × 1507 × GA21 maize and three sub combinations independently of their origin)

Minority Opinion

J.M. Wal, Member of the EFSA GMO Panel

Summary

Application (AP) 86 includes the four-event stack Bt11 x MIR162 x 1507 x GA21 maize and three subcombinations derived from this stack independently of their origin. Other subcombinations are out of the scope of the AP. According to the European Union (EU) regulation, the adoption of the four-event stack Bt11 x MIR162 x 1507 x GA21 maize will, automatically and simultaneously, result in the adoption of the three subcombinations. This will apply if they are present by natural segregation during the cultivation of the authorised four-event stack or if they are produced on their own in the future by targeted conventional breeding techniques, using maize lines different of those used and assessed in the present AP, and imported in the EU as independent stacks. No specific data regarding any of those three subcombinations have been provided by the Applicant, who also did not clearly justify why it considers that they are not necessary for the risk assessment.

The reason of this minority opinion (MO) is that a risk assessment done without specific data and based on assumptions and indirect considerations extrapolated from data obtained with the single events, the four-event stack and subcombinations previously submitted and assessed cannot be considered as strong and reliable as a comprehensive one based on a complete set of data, particularly in the present case when potential unintended adverse effects resulting in an uncontrolled risk for the health of human consumers in certain segments of the population may be expected.

For the three subcombinations, for which no experimental data were provided, the GMO Panel assessed the likelihood of interactions among the single events and the newly expressed (NEP) proteins; it concluded that they are unlikely and that their combinations would not raise safety concerns. The three maize subcombinations are therefore expected to be as safe as the single events, the previously assessed subcombinations and the four-event stack maize. The GMO Panel thus considers that neither additional data nor post-market monitoring is necessary for a comprehensive assessment of Bt11 x MIR162 x 1507 x GA21 and its three subcombinations.

One may notice that this situation is similar to that of AP 99 i.e. Bt11 x 59122 x MIR604 x 1507 x GA21 maize for which a MO was expressed for the same reasons on 15 July 2016. Therefore, the present MO is mostly a reiteration of this previous one, emphasising that unfortunately nothing has changed during the past 2 years to address this issue. However, a reflexion has been conducted by the Panel to try to establish a coherent frame for the assessment of identified intended/non-intended effects in subcombinations. This resulted in general proposals that may be applied in this case provided that the Panel clarifies the assumptions made in its assessment and the uncertainty of its approach. Unfortunately, this reflexion did not consider the important issue of the impact of the genetic background of the maize lines used which may impact on the outcomes of the assessment. Finally, no additional aspects or new considerations have been taken into account for the safety assessment of AP 86 as compared to that of AP 99 and in particular to clarify and reduce the uncertainty of the potential specific risk of those three subcombinations if they are to be produced in or imported to the EU in the future. Risk management measures that were recommended in the Scientific Opinion on AP 99 for following up the subcombinations in the case they are produced have even not been reiterated in the present one.

Presentation of AP 86

The intended use of the four-event stack is to confer insect resistance and protection against specific lepidopteran e.g. *Ostrinia nubilalis* and *Sesamia nonagrioides*.

The applicant notified EFSA that the scope of EFSA-GMO-DE-2010-86 was limited to Bt11 x MIR 162 x 1507 x GA21 maize and its three subcombinations (independently of their origin), that have never been assessed by EFSA and that may have even not been produced yet. These three subcombinations are the following: Bt11 x MIR162 x 1507; Bt11 x MIR162 x 1507; MIR162 x 1507

For any of these three subcombinations, no specific data and no clear explanation regarding why these missing experimental data were considered not necessary for the safety assessment have been provided by the Applicant. Regarding the four-event stack, it is also noteworthy that the Applicant did

not provide data on the levels of expression of the NEPs in forage as required by the Panel and that the data on the concentrations in the seeds were collected from a field trial performed in only one location; however, the significance of these results is not the actual matter of this MO.

The single events: **Bt11** (Cry1Ab protein, PAT protein); **MIR162** (Vip3Aa20 protein, PMI (Phospho Mannose Isomerase)); **1507** (Cry1F protein, PAT protein); **GA21** (mEPSPS protein) as well as four subcombinations of two-event stack maize and two of three-event stack maize that are not in the scope of this application, were previously assessed by EFSA.

Food/Feed safety assessment of AP 86

The comparative analysis of the four-event stack with the conventional counterpart did not identify differences that would raise concerns regarding the safety or nutritional value for humans and animals. They were considered not relevant for Food and Feed safety and no further assessment was required.

EFSA has previously performed a toxicological assessment of the NEPs individually in the context of the single event applications and no safety concerns were identified. The three insecticidal Bt proteins (i.e. Cry1Ab, Vip3Aa20 and Cry1F protein), the herbicide tolerant proteins (i.e. PAT and mEPSPS) and the marker protein PMI were considered unlikely to present a health risk to humans or animals and no further assessment was required.

As already noted and discussed during the assessment of AP 99, it is noteworthy that Bt proteins such as Cry1Ab and those structurally and functionally similar have a quite long history of use and are generally considered safe for mammals because of their specific mode of action as insecticide (i.e. high-affinity binding to specific receptors of insect gut mucosa). However, side effects have been observed that may affect the immune system following certain conditions of exposure. In particular, a systemic and mucosal adjuvant activity has been reported in mice after high-dose administrations of Cry1Ac by the intragastric, intraperitoneal and intranasal routes. Among other effects, an antibody response against an unrelated protein has been observed showing and adjuvant effect of Cry1Ac on the immune response (for a review, see 1). Because of these characteristics, Cry proteins such as Cry1Ac are being proposed as mucosal adjuvant for increasing the efficacy of vaccination.

This issue is in relation with the doses of administration although very little is known regarding the dose–response relationship and the manifestation of such an adjuvant activity in other Bt proteins than Cry1Ab and Cry1Ac. It has been shown by different research groups that (i) at the dose at which it is expressed in MON 810, Cry1Ab did not exert an adjuvant activity (at least for the MON 810 cultivars that have been tested) and (ii) the adjuvant activity would anyway differ from that of cholera toxin by its mode of action, which was at this time a major concern expressed by some Member State Competent Authorities (for a review, see 2).

[For information, it is now thought to be due to a dose-related effect on the innate immunity system but information on the specific activity and possible interactions or additivity of the different Bt proteins are lacking.]

At this time, it was then concluded that, in those single events, the risk of adverse effects because of an adjuvant activity was unlikely.

In the present four-event stack, the levels of expression of the NEPs are similar to those measured in the singles. Given the low concentrations of the three Bt proteins, it may be assumed from the literature that the manifestation of an adjuvant activity by their presence and combination is unlikely even in the case of a possible additive effect. This conclusion also pertains to the subcombinations derived from the four-event stack by natural segregation during its cultivation.

There is thus no disagreement with the scientific opinion of the GMO Panel regarding the four-event stack and the subcombinations derived from this stack by natural segregation. The disagreement results from the conclusion on the three 'sub-combinations independently of their origin' for which no data are available. Some of these subcombinations may already exist, but they should mainly be produced in the future by targeted conventional breeding possibly with maize lines different from those used in the four-event stacks, the single events and subcombinations previously assessed. The Panel expects that the concentrations of the newly expressed Bt proteins will be similar to those actually measured in the present four-event stack and in the other applications. It made no reservation regarding higher expressions levels that could occur, presently or in the future in different conditions of breeding, assuming that the variability of the expression levels due to environmental conditions will remain low and implicitly not taking into account a possible impact of the genetic background on the variability of the levels of NEPs.

Therefore, it considers, according to the General Strategy for the risk assessment of subcombinations in segregating stacks (3), that no further data are needed to conclude on the safety

of the subcombinations. Indeed, unintended effects on the immune system have never been identified in any application where Bt proteins were expressed; but at the same time, it should also be noted that they could not be observed by the toxicological studies (i.e. 28-day repeated-dose tox studies and/or 90-day feeding trials) currently recommended and performed for the safety assessment of GM plants at EFSA because they do not include appropriate tests for this purpose.

As a consequence of the conclusion of the scientific opinion, even in the absence of actual compositional data, stacks eventually produced in the future would be *de facto* authorised and could be imported in the EU without any further assessment or possible post-marketing programme or any other restrictive risk management measures as recommended in the same situation for AP99.

However, compositional data and reliable information on the actual concentrations of the NEPs are crucial to achieve a sound safety assessment. Indeed, it has been shown that the genetic background of the recipient plant has a major effect on Cry1Ac expression in GM cotton (4) and maize (5, 6); it may cause an additional variability (not taken into account by the GMO Panel so far) in Bt protein concentrations which might impact on the safety. The risk of increased expression of the newly expressed Bt proteins and of a possible cumulative effect of their combination on the immune system (e.g. resulting in an adjuvant activity) cannot be ruled out although it is difficult to evaluate in the absence of actual experimental data. Indeed, the scope of AP 86 is for import and processing which suggests a limited exposure for consumers in the EU. Nevertheless, should those subcombinations (or some of them) be produced and commercialised in the future, the resulting risk for human health, particularly in workers, might be higher than that of singles or of the fully assessed Bt11 x MIR162 x 1507 x GA21 maize.

Conclusion

It is not acceptable that the same weight and reliability are given to the assessment of a GM crop for which a complete data set is available and can be comprehensively evaluated and to GM crops for which no specific data are provided, particularly when there is a health concern resulting from a possible over expression of the NEPs.

In the case of AP 86, the safety assessment of the three subcombinations only relies on the data from the four-event stack, from the single events and from subcombinations previously assessed in other applications. Despite missing data on comparative analysis, the GMO Panel concludes that it expects no adverse effect on human health based on an extrapolation. The criteria and procedure used are not detailed and remain mostly implicit; the level of confidence that should be required for this extrapolation is not given and there is no critical appraisal of its limitations. No evaluation of the resulting uncertainty has been performed. These weaknesses may invalidate the conclusion which anticipates the absence of safety concerns and does not require that additional specific data shall be provided to EFSA to guarantee the safety of these three subcombinations should they be produced and imported to the EU market in the future.

This MO is not only a question of principle since a risk for the human health may result from a possible over expression of NEPs due to the different factors of variability already mentioned if those subcombinations were to be produced. Hopefully the risk might be low, but the uncertainty could be much decreased if sufficient specific data were provided by the Applicant before all 'sub-combinations independently of their origin' are approved.

Allergic reactions in general and particularly food allergy are dramatically increasing in the EU (and worldwide) and have become a most important public health issue. The reasons are unclear, but most specialists involve the changes in environmental conditions, in cultivated plant species and in food habits. Indeed, environmental conditions are known to play a major role in the occurrence and/or severity of the allergic reaction in addition to the genetic background of predisposed individuals and characteristics of the allergen. They include the route and doses of exposure to the protein in question but also the presence in the food/diet of compounds known to modulate (e.g. increase) the immune response to other unrelated proteins present in the food. The potential role of these 'adjuvants' is therefore emphasised and especially in the case of immunoglobulin E (IgE)-mediated allergy.

It is thus a pity that a high-double uncertainty due to both a lack of knowledge and a lack of data, still remains which clarification would improve the assessment, clarify the role/absence of role of GMOs in the increasing allergenic risk and finally allow a solid protection and prevention of at risk consumers.

One solution is at least to reduce the uncertainty on the levels of expression of NEPs by measuring and collecting actual reliable data for each subcombination through well-designed field trials and taking into account all the factors of variability including the genetic background of varieties used for producing the subcombinations/stacks.

The second is to develop and perform methods that would allow identifying unintended effects that are known and expected. In the present case, a better knowledge of the mechanisms of action of Bt proteins for a biological activity that coexists with the intended function (e.g. effect of the different Bt proteins on the immune system vs. insecticide activity) and of the dose–(side) effect relationship would also allow decreasing the uncertainty of the risk assessment. Such methods adapted for the identification of potential effects on the immune response are available and are already performed. They are based on the administration of repeated doses of the NEP to well-known appropriate animal models (review in 1, 7, 8, 9) and/or even administration of the whole GM diet in the context of adapted 90-day feeding studies (10), then measuring a set of biomarkers of immunity and of the antibody response in blood samples collected at different time points.

So far, none of these studies has demonstrated significant/relevant adverse effects in the conditions they were performed. That is why, it is most important to continue performing such studies even if they are not fully validated in order to hopefully discard any increased allergenic risk due to GMOs with stronger arguments than implicit and highly uncertain assumptions.

Should the present situation remain, this might increase consumers questioning, fear and rejection of GMOs.

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