SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 15 July 2016

doi: 10.2903/j.efsa.2016.4567

Scientific Opinion on an application by Syngenta (EFSA-GMO-DE-2011-99) for the placing on the market of maize Bt11 × 59122 × MIR604 × 1507 × GA21 and twenty subcombinations, which have not been authorised previously independently of their origin, for food and feed uses, import and processing under Regulation (EC) No 1829/2003

EFSA Panel on Genetically Modified Organisms (GMO)

Abstract

In this opinion, the EFSA GMO Panel assesses the five-event stack maize and 20 of its subcombinations independently of their origin. The EFSA GMO Panel has previously assessed the five single events that are combined to produce this five-event stack maize Bt11 × 59122 × MIR604 × 1507 × GA21 and did not identify safety concerns. No new data on the single events, leading to a modification of the original conclusions on their safety, were identified. The molecular, agronomic, phenotypic and compositional data on the five-event stack maize did not give rise to safety concerns and there is no reason to expect interactions between the single events impacting on the food and feed safety of the five-event stack maize. Considering the scope of the application (no cultivation), routes of exposure and limited exposure levels, the Panel concludes that this five-event stack maize would not raise safety concerns in the event of accidental release of viable grains into the environment. The EFSA GMO Panel concludes that the five-event stack maize is as safe and as nutritious as its conventional counterpart in the context of its scope. For the 20 subcombinations, the EFSA GMO Panel followed a weight-of-evidence approach, and concluded that they are expected to be as safe as the five-event stack maize. No specific data were submitted for the subcombinations included in the scope of this application that could be produced by conventional crossing through targeted breeding approaches. In order to reduce the consequent uncertainties and to confirm assumptions made for their assessment, the EFSA GMO Panel considers that the applicant should provide relevant information, if these subcombinations were to be created via targeted breeding approaches and imported into the EU in the future. In this case, this information should focus on expression levels of the newly expressed proteins.
A minority opinion expressed by an EFSA GMO Panel member is appended to this opinion.

Keywords: GMO, maize (*Zea mays*), maize Bt11 × 59122 × MIR604 × 1507 × GA21, insect resistant and herbicide tolerant, Regulation (EC) 1829/2003

Requestor: Competent Authority of Germany

Question number: EFSA-Q-2011-00894

Correspondence: GMO@efsa.europa.eu

Panel members: Andrew Nicholas Birch, Josep Casacuberta, Adinda De Schrijver, Mikolaj 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 A)

Acknowledgements: The Panel wishes to thank the members of its standing Working Groups on Molecular Characterisation, Food/Feed and Environmental Risk Assessment for the preparatory work on this scientific opinion, and EFSA staff members: Hermann Broll, Andrea Gennaro and Irina Olaru for the support provided to this scientific opinion.

Suggested citation: EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2016. Scientific Opinion on an application by Syngenta (EFSA-GMO-DE-2011-99) for the placing on the market of maize Bt11 × 59122 × MIR604 × 1507 × GA21 and twenty subcombinations, which have not been authorised previously independently of their origin, for food and feed uses, import and processing under Regulation (EC) No 1829/2003. EFSA Journal 2016;14(8):4567, 31 pp. doi:10.2903/j.efsa.2016.4567

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Summary

Following the submission of application EFSA-GMO-DE-2011-99 under Regulation (EC) No 1829/2003 from Syngenta, the Panel on Genetically Modified Organisms of the European Food Safety Authority (hereafter referred as EFSA GMO Panel) was asked to deliver a scientific opinion on the safety of herbicide-tolerant and insect-resistant genetically modified maize Bt11 × 59122 × MIR604 × 1507 × GA21 (referred to hereafter as 'five-event stack maize') and 20 subcombinations (referred to as 'subcombinations independently of their origin' in line with the Commission implementing regulation (EU) No 503/2013). The scope of application EFSA-GMO-DE-2011-99 is for food and feed uses, import and processing, but excludes cultivation within the European Union (EU).

The term 'subcombination' refers to any combination of up to four of the events present in the five-event stack maize Bt11 × 59122 × MIR604 × 1507 × GA21. Subcombinations occur as segregating progeny in the harvested grains of Bt11 × 59122 × MIR604 × 1507 × GA21 (embryo and albumen), and their safety is evaluated within the assessment of the five-event stack maize Bt11 × 59122 × MIR604 × 1507 × GA21 in Section 3.4 of the present opinion.

'Subcombination' also refers to any combination of up to four of the events Bt11, 59122, MIR604, 1507 or GA21 that has either been or could be produced by conventional crossing, through targeted breeding approaches (EFSA GMO Panel, 2011). These are maize stacks that can be bred, produced and marketed independently of the five-event stack maize Bt11 × 59122 × MIR604 × 1507 × GA21. These stacks, including their segregating progeny, are risk assessed in Section 3.5 of the present opinion.

In accordance with the EFSA GMO Panel guidance document applicable to this application (EFSA, 2006, 2007a), 'where all single events have been assessed, the risk assessment of stacked events should focus mainly on issues related to (a) stability, (b) expression of the events and (c) potential interactions between the events'. For application EFSA-GMO-DE-2011-99, previous assessments of the five single events (Bt11, 59122, MIR604, 1507 and GA21) provided a basis to evaluate the five-event stack maize and the 20 subcombinations.

The five-event stack maize Bt11 × 59122 × MIR604 × 1507 × GA21 was produced by conventional crossing to combine five single maize events. Maize containing the single events, Bt11 (expressing Cry1Ab and PAT proteins), 59122 (expressing Cry34Ab1, Cry35Ab1 and PAT proteins), MIR604 (expressing mCry3A and PMI proteins), 1507 (producing Cry1F and PAT proteins) and GA21 (expressing mEPSPS protein), were assessed previously and no concerns were identified. No safety issue was identified by updated bioinformatic analyses nor reported by the applicant concerning the five single maize events, since the publication of the scientific opinions. Consequently, the EFSA GMO Panel considers that its previous conclusions on the safety of the single maize events remain valid (Section 3.2).

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

The molecular data establish that the transformation events stacked in maize Bt11 × 59122 × MIR604 × 1507 × GA21 have the same molecular properties and characteristics as the single transformation events. Protein expression analyses showed that the levels of the newly expressed proteins are similar in the five-event stack and the single events, with the exception of PMI.

Comparison of the levels of the newly expressed proteins between the five-event stack and the respective single events did not reveal an interaction that would affect protein expression level.

The newly expressed proteins in the five-event stack maize did not raise concerns for human and animal health. The compositional data indicate that maize Bt11 × 59122 × MIR604 × 1507 × GA21 would be expected to deliver the same nutritional characteristics as its conventional counterpart. This was confirmed by the results of an animal feeding study in chickens for fattening.

The Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) considers that there is no reason to expect interactions that could impact on the food and feed safety. No safety concerns are foreseen for any of the 20 subcombinations not previously assessed by EFSA.

Considering the combined events, the outcome of the comparative analysis, the routes of exposure and the limited exposure levels, the EFSA GMO Panel concluded that this five-event stack maize would

not raise safety concerns in the event of accidental release of viable GM maize grains into the environment.

In conclusion, the EFSA GMO Panel is of the opinion that the five-event stack maize is as safe and as nutritious as its conventional counterpart and non-GM commercial maize varieties in the context of its scope.

No scientific information regarding the subcombinations covered in the scope of this application was retrieved in a literature search covering the period since the publication of the respective scientific opinions.

The EFSA GMO Panel did not find indication that the subcombinations, resulting from combination of any of the single events included in the five-event stack maize, would raise safety concerns. However, for all 20 subcombinations (Table 1) that could be produced by conventional crossing through targeted breeding approaches, no specific data were submitted. For these, the EFSA GMO Panel has drawn conclusions on a weight-of-evidence approach, which identified uncertainties due to data gaps. In order to reduce these uncertainties and to confirm assumptions made for the assessment of these subcombinations, the EFSA GMO Panel considers that the applicant should provide relevant information, if these subcombinations were to be created via targeted breeding approaches and imported into the EU in the future. In this case, this information should focus on expression levels of the newly expressed proteins.

The EFSA GMO Panel considers that post-market monitoring of GM food/feed is not necessary, given the absence of safety concerns identified for the five-event stack maize and the 20 lower subcombinations. If these subcombinations were to be created via targeted breeding approaches and imported into the EU, the requirement for monitoring should be considered on the basis of the new protein expression data provided.

The EFSA GMO Panel is of the opinion that the PMEM plans provided by the applicant are in line with the scope of the five-event stack maize and the 20 subcombinations.

In delivering its scientific opinion, the EFSA GMO Panel considered the data available on the five-event stack maize and the single events, the scientific comments submitted by the Member States and the relevant scientific publications.

A minority opinion expressed by an EFSA GMO Panel member is presented in the Appendix A of this opinion.

Table of contents

1. Introduction

1.1. Background

On 7 July 2011, the European Food Safety Authority (EFSA) received from the Competent Authority of Germany an application EFSA-GMO-DE-2011-99 for authorisation of genetically modified (GM) maize Bt11 × 59122 × MIR604 × 1507 × GA21 (referred to hereafter as five-event stack maize), submitted by Syngenta Crop Protection AG (referred to hereafter as the applicant) within the framework of Regulation (EC) No 1829/2003[1], for food and feed uses, import and processing. Subsequently, the applicant requested twice, EFSA to consider a modification in the scope of application EFSA-GMO-DE-2011-99. The risk assessment presented here is for application EFSA-GMO-DE-2011-99 for the placing on the market of GM maize Bt11 × 59122 × MIR604 × 1507 × GA21 and subcombinations that have not been authorised previously (Table 1), independently of their origin, for food and feed uses, import and processing.

After receiving the application EFSA-GMO-DE-2011-99 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. 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 21 December 2011 and 2 April 2012, EFSA received additional information (requested on 17 August 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 the Member States and the European Commission, and consulted nominated risk assessment bodies of the Member States, including the national Competent Authorities within the meaning of Directive 2001/18/EC[2] following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. The Member States had 3 months after the date of receipt of the valid application (until 18 September 2012) to make their opinion known.

The EFSA GMO Panel carried out an evaluation of the scientific risk assessment of the five-event stack maize and subcombinations that have not been authorised previously (Table 1) (referred to as 'subcombinations independently of their origin' according to the Commission Implementing Regulation (EU) No 503/2013[3]). On 3 March 2013, 23 June 2014, 30 September 2014, 21 November 2014, 27 July 2015, 24 September 2015 and 4 April 2016, EFSA received additional information (requested on 7 December 2012, 5 February 2013, 23 September 2013, 12 March 2014, 27 October 2014, 19 September 2015 and 23 December 2015, respectively). The applicant provided additional information spontaneously on 28 July 2014, 21 July 2015 and 17 December 2015.

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 EFSA 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.

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 EFSA GMO Panel was requested to carry out a scientific risk assessment of 'maize Bt11 × 59122 × MIR604 × 1507 × GA21 and twenty subcombinations of the single events (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.

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

[2] 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.

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

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 EFSA GMO Panel was not requested to give an opinion on information required under Annex II to the Cartagena Protocol. Furthermore, the EFSA 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 the application EFSA-GMO-DE-2011-99, 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 × 59122 × MIR604 × 1507 × GA21 and 20 subcombinations that have not been authorised previously, independently of their origin (Table 1), 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, 2011), for the environmental risk assessment (ERA) of GM plants (EFSA GMO Panel, 2010) and for the post-market environmental monitoring (PMEM) of GM plants (EFSA, 2011a).

The comments raised by the Member States are addressed in Annex G of EFSA's overall opinion and were taken into consideration during the scientific risk assessment.[4]

3. Assessment

3.1. Introduction

This application EFSA-GMO-DE-2011-99 covers 21 events: the five-event stack maize Bt11 × 59122 × MIR604 × 1507 × GA21 and 20 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 20 combinations of up to four of the events present in the five-event stack maize. Subcombinations occur as segregating progeny in harvested grains of the five-event stack maize, and their safety is part of the assessment of the five-event stack maize in Section 3.3 of this EFSA GMO Panel Scientific Opinion.

'Subcombination' also refers to combinations of four, three or two of the five events Bt11, 59122, MIR604, 1507 and GA21 that have either been or could be produced by conventional crossing through targeted breeding approaches (EFSA GMO Panel, 2011). These are maize stacks that can be bred, produced and marketed independently of the five-event stack maize. These subcombinations, except for four two-event stack maize events and a three-event stack maize event, that are not in the scope of this application, are risk assessed in Section 3.4 of this EFSA GMO Panel Scientific Opinion.

The five-event stack maize was developed to achieve insect resistance and herbicide tolerance to glyphosate- and glufosinate ammonium-based herbicides. The insect resistance confers protection against specific lepidopteran (e.g. *Ostrinia nubilalis* (European corn borer) and *Sesamia nonagrioides* (Mediterranean corn borer)) and coleopteran pests (*Diabrotica* spp. (corn rootworm larvae)).

[4] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2011-00894

Table 1: Twenty-one maize events covered by the scope of the application EFSA-GMO-DE-2011-99

Degree of stacking	Events	Unique identifiers
Five-event stack maize	Bt11 × 59122 × MIR604 × 1507 × GA21	SYN-BTØ11-1 × DAS-59122-7 × SYN-IR6Ø4-5 × DAS-Ø15Ø7-1 × MON-ØØØ21-9
Four-event stack maize	Bt11 × MIR604 × 1507 × GA21	SYN-BTØ11-1 × SYN-IR6Ø4-5 × DAS-Ø15Ø7-1 × MON-ØØØ21-9
	Bt11 × 59122 × 1507 × GA21	SYN-BTØ11-1 × SYN-IR162-4 × DAS-Ø15Ø7-1 × MON-ØØØ21-9
	Bt11 × 59122 × MIR604 × GA21	SYN-BTØ11-1 × DAS-59122-7 × SYN-IR6Ø4- × MON-ØØØ21-9
	Bt11 × 59122 × MIR604 × 1507	SYN-BTØ11-1 × DAS-59122-7 × SYN-IR6Ø4-5 × DAS-Ø15Ø7-1
	59122 × MIR604 × 1507 × GA21	DAS-59122-7 × SYN-IR6Ø4-5 × DAS-Ø15Ø7-1 × MON-ØØØ21-9
Three-event stack maize	Bt11 × 59122 × MIR604	SYN-BTØ11-1 × DAS-59122-7 × SYN-IR6Ø4-5
	Bt11 × 59122 × 1507	SYN-BTØ11-1 × DAS-59122-7 × Ø15Ø7-1
	Bt11 × 59122 × GA21	SYN-BTØ11-1 × DAS-59122-7 × MON-ØØØ21-9
	Bt11 × MIR604 × 1507	SYN-BTØ11-1 × SYN-IR6Ø4-5 × DAS-Ø15Ø7-1
	Bt11 × 1507 × GA21	SYN-BTØ11-1 × DAS-Ø15Ø7-1 × MON-ØØØ21-9
	59122 × MIR604 × 1507	DAS-59122-7 × SYN-IR6Ø4-5 × DAS-Ø15Ø7-1
	59122 × MIR604 × GA21	DAS-59122-7 × SYN-IR6Ø4-5 × MON-ØØØ21-9
	59122 × 1507 × GA21	DAS-59122-7 × DAS-Ø15Ø7-1 × MON-ØØØ21-9
	MIR604 × 1507 × GA21	SYN-IR6Ø4-5 × DAS-Ø15Ø7-1 × MON-ØØØ21-9
Two-event stack maize	Bt11 × 59122	SYN-BTØ11-1 × DAS-59122-7
	Bt11 × 1507	SYN-BTØ11-1 × DAS-Ø15Ø7-1
	59122 × MIR604	DAS-59122-7 × SYN-IR6Ø4-5
	59122 × GA21	DAS-59122-7 × MON-ØØØ21-9
	MIR604 × 1507	SYN-IR6Ø4-5 × DAS-Ø15Ø7-1
	1507 × GA21	DAS-Ø15Ø7-1 × MON-ØØØ21-9

All five single maize events Bt11, 59122, MIR604, 1507 and GA21 and five of the maize stacks have been previously assessed (see Table 2). No concerns for human and animal health, or environmental safety were identified.

Table 2: Single maize events and maize stacks already assessed by the EFSA GMO Panel

Events	Application or mandate	EFSA Scientific Opinions
Bt11	C/F/96/05.10	2005a
	EFSA-GMO-RX-Bt11	2009a
	EFSA-M-2012–0232[(a)]	2012b
59122	EFSA-GMO-NL-2005-12	2007c
	EFSA-GMO-NL-2005-23	2013
MIR604	EFSA-GMO-UK-2005-11	2009d
1507	C/NL/00/10	2004
	C/ES/01/01	2005b
	EFSA-GMO-RX-1507	2009c
GA21	EFSA-GMO-UK-2005-19	2007b
	EFSA-GMO-RX-GA21	2007b
	EFSA-GMO-UK-2008-60	2011b
59122 × 1507	EFSA-GMO-NL-2005-15	2009b
MIR604 × GA21	EFSA-GMO-UK-2007-48	2010a
	EFSA-GMO-DE-2009-66	2015a

Events	Application or mandate	EFSA Scientific Opinions
Bt11 × GA21	EFSA-GMO-UK-2007-49	2009e
	EFSA-GMO-DE-2009-66	2015a
Bt11 × MIR604	EFSA-GMO-UK-2007-50	2010b
	EFSA-GMO-DE-2009-66	2015a
Bt11 × MIR604 × GA21	EFSA-GMO-UK-2008-56	2010c
	EFSA-GMO-DE-2009-66	2015a

EFSA GMO Panel: Panel on Genetically Modified Organisms of the European Food Safety Authority.
(a): Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2012-00713

The EFSA 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, (b) expression of the events and (c) potential interactions between the events' (EFSA, 2007a; EFSA GMO Panel, 2011).

3.2. Updated information on single events

Since the publication of the scientific opinions on the single maize events by the EFSA GMO Panel (EFSA, 2004, 2005a, 2007b, 2009b; EFSA GMO Panel, 2013), no safety issue pertaining to the five single events has been reported by the applicant.

For events MIR604 and GA21, updated nucleotide sequence information was received.[5] In the case of event MIR604, a single nucleotide difference was identified in the non-coding region of the insert as compared with the sequence originally reported in 2005. Further analyses demonstrated that this nucleotide difference had already been present in the original material used for the risk assessment of maize MIR604. In the case of event GA21, 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* (5-enolpyruvylshikimate-3-phosphate synthase) cassettes present within the insert. Similar to event MIR604, further analyses demonstrated that these differences had already been present in the original material used for the risk assessment of maize GA21. The EFSA GMO Panel has performed the risk assessment of the new sequencing information for events MIR604 and GA21 in the frame of a request received from the European Commission[6] and concluded that the original risk assessments of events MIR604 and GA21 as a single and as a part of stacked events remains valid (EFSA GMO Panel, 2015b,c).

Bioinformatic analyses on the junction regions for events Bt11, 59122, MIR604, 1507 and GA21, using the most up-to-date nucleotide sequences and methodology specified in the EFSA 2011 guidance (EFSA GMO Panel, 2011), confirmed that no known endogenous genes were disrupted by any of the inserts.[7]

Updated bioinformatic analyses of the amino acid sequence of the newly expressed Cry1Ab, Cry34Ab1, Cry35Ab1, mCry3A, Cry1F, PAT, PMI and mEPSPS proteins revealed no significant similarities to toxins and allergens.[7] In addition, updated bioinformatics analyses of the newly created open reading frames (ORFs) within the inserts and at their junctions, indicate that the expression of an ORF showing significant similarities to toxins or allergens is highly unlikely.[8]

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

3.3. Risk assessment of the five-event stack maize Bt11 × 59122 × MIR604 × 1507 × GA21

3.3.1. Molecular characterisation

Possible interactions affecting the integrity of the single events, protein expression level or the biological function conferred by the individual inserts are considered below.

[5] Additional information: 21/7/2015, 24/9/2015.
[6] EFSA-Q-2015-00473 and EFSA-Q-2015-00475.
[7] Additional information: 30/7/2015.
[8] Additional information: 30/7/2015, 17/12/2015 (spontaneous submission), 4/4/2016.

3.3.1.1. Genetic elements and their biological function

Maize Bt11, 59122, MIR604, 1507 and GA21 are combined by conventional crossing to produce the five-event stack maize Bt11 × 59122 × MIR604 × 1507 × GA21. The structures of the inserts introduced into the five-event stack maize are described in detail in the respective EFSA scientific opinions and no new genetic modifications were involved. Genetic elements in the expression cassettes of the single events are summarised in Table 3.

Intended effects of the inserts in maize Bt11 × 59122 × MIR604 × 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 Cry proteins.

Table 3: Genetic elements in the expression cassettes of the events stacked in maize Bt11 × 59122 × MIR604 × 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 (*Z. mays*)	No	*pat* (*Streptomyces viridochromogenes*)	*nos* (*A. tumefaciens*)
59122	*ubi*ZM1 (*Z. mays*)	–	No	*cry34Ab1* (*B. thuringiensis*)	*pin*II (*Solanum tuberosum*)
	Wheat peroxidase (*Triticum aestivum*)	–	No	*cry35Ab1* (*B. thuringiensis*)	*pin*II (*S. tuberosum*)
	35S (CaMV)	–	No	*pat* (*S. viridochromogenes*)	35S (CaMV)
MIR604	MTL (*Z. mays*)	–	No	m*cry3A* (*B. thuringiensis*)	*nos* (*A. tumefaciens*)
	ZmUbiInt (*Z. mays*)	–	No	*pmi* (*Escherichia coli*)	*nos* (*A. tumefaciens*)
1507[(a)]	*ubi*ZM1 (*Z. mays*)	–	No	*cry1F* (*B. thuringiensis*)	ORF25PolyA (*A. tumefaciens*)
	35S (CaMV)	–	No	*pat* (*S. viridochromogenes*)	35S (CaMV)
GA21	Actin 1 (*Oryza sativa*)	Actin 1 (*O. sativa*)	OTP (*Helianthus annuus*)	m*epsps* (*Z. mays*)	*nos* (*A. tumefaciens*)

CaMV: cauliflower mosaic virus; UTR: untranslated region.
(*): Source of genetic information.
(–): When no element was specifically introduced to optimise expression.
(a): Maize 1507 also contains 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 × 59122 × MIR604 × 1507 × GA21

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

Event	Protein	Donor organism and biological function	Intended effects in GM plant
59122	Cry34Ab1	Based on a gene from *B. thuringiensis* strain PS149B1. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (cry) genes (Schnepf et al., 1998; Ellis et al., 2002)	Event 59122 expresses a cry34Ab1 gene which was modified to enhance expression in plants. The amino acid sequence was not modified. Cry34Ab1 is a protein toxic to certain coleopteran larvae feeding on maize
	Cry35Ab1	Based on a gene from *B. thuringiensis* strain PS149B1. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes (Schnepf et al., 1998; Ellis et al., 2002)	Event 59122 expresses a cry35Ab1 gene which was modified to enhance expression in plants. The amino acid sequence was not modified. Cry35Ab1 is a protein toxic to certain coleopteran larvae feeding on maize
	PAT	Based on a gene from *S. viridochromogenes*. PAT enzyme confers resistance to the antibiotic bialaphos (Wohlleben et al., 1988)	PAT acetylates L-glufosinate-ammonium and thereby confers tolerance to glufosinate ammonium-based herbicides
MIR604	mCry3A	Based on a gene from *B. thuringiensis* subsp. *tenebrionis* (Sekar et al., 1987). *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes (Schnepf et al., 1998)	The N-terminal 48 amino acid residues of the native Cry3A protein were deleted. In addition, a cathepsin-G protease recognition site was introduced for enhanced efficiency towards target pests (Chen and Stacy, 2003). Cry3A is a protein toxic to certain coleopteran larvae feeding on maize
	PMI	Based on a gene from *Escherichia coli*. Phosphomannose isomerase (PMI) catalyses the isomerisation of mannose-6-phosphate to fructose-6-phosphate and plays a role in the metabolism of mannose (Markovitz et al., 1967)	PMI is used as a selectable marker in maize MIR604. 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	Based on a gene from *B. thuringiensis* subsp. *aizawai*. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes (Schnepf et al., 1998)	Event 1507 expresses a synthetic version of the truncated Cry1F protein. Cry1F is a protein toxic to certain lepidopteran larvae feeding on maize
	PAT	Based on a gene from *S. viridochromogenes* strain Tü494. PAT enzyme confers resistance to the antibiotic bialaphos (Wohlleben et al., 1988)	Event 1507 expresses a synthetic version of the PAT protein, which confers tolerance to glufosinate ammonium-based herbicides
GA21	mEPSPS	Based on a gene from *Zea mays* 5-enolpyruvylshikimate-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)

GM: genetically modified.

3.3.1.2. Integrity of the events in the five-event stack maize Bt11 × 59122 × MIR604 × 1507 × GA21[9]

The genetic stability of the inserted DNA over multiple generations in the five single maize events was demonstrated previously (EFSA, 2004, 2005a, 2007b,c, 2009b). Integrity of these events was demonstrated in the five-event stack maize by Southern analyses.[10]

3.3.1.3. Information on the expression of the inserts[11]

Plants were grown at a single location (five replicate blocks) under field conditions in 2009 in the USA. The levels of Cry1Ab, Cry34Ab1, Cry35Ab1, mCry3A, Cry1F, PAT, PMI and mEPSPS proteins in the five-event stack maize and the five single events were quantified by enzyme-linked immunosorbent

[9] Dossier: Part I – Section D5.
[10] Dossier: Part I – Section D5 and Appendix 3.
[11] Dossier: Part I – Section D3.

assay (ELISA). Protein levels were determined in leaves (V5 stage), root (R1 stage), pollen (R1 stage), whole plant (R1 stage) and grain (R6 stage). Only data on grain at physiological maturity is reported below (Table 5).

Interactions between events may result in changes of expression of the newly expressed proteins. Cry1Ab, Cry34Ab1, Cry35Ab1, mCry3A, Cry1F, PAT, PMI and mEPSPS proteins in the five-event stack maize were comparable to the corresponding levels in the single maize events and showed no changes that could be the result of such interactions (see Table 5 for protein levels in grain).

Table 5: Means (upper row) and ranges (lower row) of protein levels (μg/g dry weight) in grain at physiological maturity from the single maize events Bt11, 59122, MIR604, 1507, GA21 and the five-event stack maize

Protein	Bt11 × 59122 × MIR604 × 1507 × GA21	Bt11	59122	MIR604	1507	GA21
Cry1Ab	2.08 1.64–2.64	2.17 1.58–2.86	–	–	–	–
Cry34Ab1	80.83 56.2–133.4	–	92.25 59.6–158.2	–	–	–
Cry35Ab1	1.82 1.55–2.06	–	2.02 1.80–2.57	–	–	–
mCry3A	0.42 0.34–0.50	–	–	0.42 0.35–0.57	–	–
Cry1F	2.63 1.99–3.34	–	–	–	2.74 2.23–3.75	–
PAT	0.12 0.06–0.17	LOQ	LOQ–0.09	–	LOQ	–
PMI	2.57 2.11–2.93	–	–	2.80 2.46–3.78	–	–
mEPSPS	11.88 8.97–13.28	–	–	–	–	10.89 9.15–13.27

LOQ: values below limit of quantification.
–: not assayed.

3.3.1.4. Conclusion of the molecular characterisation

The molecular data establish that the events stacked in maize Bt11 × 59122 × MIR604 × 1507 × GA21 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins are similar in the five-event stack and in the single events. Therefore, there is no indication of an interaction that may affect the integrity of the events and the levels of the newly expressed proteins in this stack.

Based on the known biological function of the newly expressed proteins, the only foreseen interactions at the biological level are between the Cry proteins in susceptible insects, which will be dealt with in Section 3.3.4.

3.3.2. Comparative assessment

3.3.2.1. Choice of comparator and production of material for the comparative assessment[12]

Two comparative field studies were performed, one for agronomic and phenotypic characterisation and one for compositional analysis.

For the analysis of agronomic and phenotypic characteristics, the five-event stack maize and its conventional counterpart were grown in 11 locations in the USA in 2009.

The conventional counterpart in these field trials was a non-GM maize line (5XH751/NP2222) with a genetic background comparable with that of maize Bt11 × 59122 × MIR604 × 1507 × GA21 (as documented by the pedigree). At each location, the test materials were grown according to a randomised complete block design with four replicates. A maintenance pesticide treatment was applied to all maize materials according to the need at each site. No treatments of the five-event stack maize

[12] Dossier: Part I – Section D7.2.

with the intended herbicides were included in the study. This experimental design allows a direct comparison between the five-event stack maize and its conventional counterpart in the absence of target herbicides.

For the compositional analysis of forage and grain, a set of field trials was performed in six locations in the USA in 2009. At each location, the following test materials (all treated with maintenance pesticides) were grown in a randomised complete block design with four replicates: the five-event stack maize, its conventional counterpart (maize 5XH751/NP2222) and the five-event stack maize additionally treated with glyphosate- and glufosinate-ammonium-based herbicides.

Additionally, in a separate field trials study,[13] eight non-GM commercially available maize lines were grown at eight sites in the USA in 2009[14] (in a randomised complete block design with four replicates), in order to establish the range of natural variation for maize compositional parameters.

Data on compositional and agronomic and phenotypic endpoints were statistically analysed for potential differences between maize Bt11 × 59122 × MIR604 × 1507 × GA21 and its conventional counterpart using the two analysis of variance (ANOVA) models: an across-site analysis (all trial sites combined) and an individual-site analysis.[15] Summary statistics of compositional data for the non-GM commercial reference varieties[13] were used for comparison, with no formal analysis.

3.3.2.2. Agronomic and phenotypic analysis[16]

The analysis of agronomic and phenotypic characteristics included 14 endpoints related to crop physiology, morphology, development and yield. Data collected for seven of the 14 endpoints were subject to ANOVA.[17] For the other seven endpoints, which couldn't be analysed by ANOVA, only summary statistics were provided.[18]

In the across-site analysis, significant differences between the five-event stack maize and its conventional counterpart were identified for early and final stand count. The mean early stand count (plants per plot) was 73.4 and 77.1 for maize Bt11 × 59122 × MIR604 × 1507 × GA21 and the conventional counterpart, respectively; the mean final stand count (plants per plot) was 71.4 and 74.9 for maize Bt11 × 59122 × MIR604 × 1507 × GA21 and the conventional counterpart, respectively.

The differences in agronomic characteristics are further assessed for their potential environmental impact in Section 3.3.4.

3.3.2.3. Compositional analysis[19]

The compositional analysis included 59 compositional parameters for grain[20] and nine for forage,[21] consistently with OECD recommendations (OECD, 2002).

Table 6 shows the results for the forage and grain parameters for which significant differences were found between maize Bt11 × 59122 × MIR604 × 1507 × GA21 and its conventional counterpart. For forage composition, a statistically significant difference for phosphorus was observed between maize Bt11 × 59122 × MIR604 × 1507 × GA21 (both treated and not treated with the target herbicides) and the conventional counterpart. For grain components, 16 significant differences with respect to the conventional counterpart were found for maize Bt11 × 59122 × MIR604 × 1507 × GA21 (untreated) and 15 statistically significant differences for maize Bt11 × 59122 × MIR604 × 1507 × GA21 (treated with target herbicides). Thirteen of the significant differences were common to both treatments.

[13] Additional information 28/7/2014/Appendix 16.
[14] York, NB; Swanton, OH; Deerfield, MI; Richland, IO; Seymour, IL; York, NB; Kimballton, IO; Elk Horn, IO.
[15] In both models, the overall mean and the genotype effect were fixed factors. The random factors were: the block effect for the individual-site analysis and the site effect, block-within-site effect and site-by-genotype interaction for the across-site analysis.
[16] Dossier: Part I – Section D7.4.
[17] The following endpoints were analysed: early stand count, ear height, plant height, final stand count, grain yield, grain moisture and test weight.
[18] The endpoints were early growth rating, days to 50% pollen shed, days to 50% silking, stay green, root lodged plants, stalk lodged plants and dropped ears.
[19] Dossier: Part I – Section D7.3.
[20] Moisture, protein, fat, ash, carbohydrates by calculation, acid detergent fibre (ADF), neutral detergent fibre (NDF), total dietary fibre (TDF), starch, calcium, copper, iron, magnesium, manganese, phosphorus, potassium, selenium, sodium, zinc, β-carotene, thiamine, riboflavin, niacin, pyridoxine, folic acid, α-tocopherol, alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, 16:0 palmitic acid, 18:0 stearic acid, 18:1 oleic acid, 18:2 linoleic acid, 18:3 linolenic acid, 20:0 arachidic acid, 20:1 eicosenoic acid, 22:0 behenic acid, ferulic acid, furfural, inositol, *p*-coumaric acid, phytic acid, raffinose and trypsin inhibitor.
[21] Proximates (moisture, crude protein, crude fat and carbohydrates by calculation), starch, fibre (ADF and NDF) and minerals (calcium and phosphorus).

The levels of carbohydrates, copper, potassium, niacin and inositol in maize Bt11 × 59122 × MIR604 × 1507 × GA21 (treated and untreated) fell within the range of variation established by the non-GM commercial maize varieties in the US 2009 field trials.[13]

For the remaining endpoints in Table 6, which fell outside the range of the commercial varieties, the EFSA GMO Panel considered the magnitude of the observed levels and the well-known biochemical roles and characteristics of the parameters, and concluded that further assessment for potential impacts on human and animal health was not required.

Table 6: Compositional endpoints (US 2009 field trials data) for which significant differences were found between maize Bt11 × 59122 × MIR604 × 1507 × GA21 and its conventional counterpart 5XH751/NP2222. For the GM maize, the conventional counterpart and the non-GM commercial varieties, the values shown are estimated means and significantly different entries are marked with an asterisk. For the non-GM commercial varieties, the range of mean values (averaged across sites) is shown

Endpoint	Conventional counterpart (5XH751/NP2222)[(a)]	Maize Bt11 × 59122× MIR604 × 1507 × GA21		Non-GM maize commercial varieties[(a),(c)]
		Untreated[(a)]	Treated[(b)]	
Forage				
Phosphorus (mg/kg dw)	1,812	2,002*	2,048*	1,763–2,034
Grain[(a)]				
Fat (% dw)	4.21	4.39*	4.36*	3.32–4.15
Carbohydrates (% dw)	84.9	84.3*	84.2*	83.6–86.1
Copper (mg/kg dw)	1.82	1.64*	1.66*	1.61–2.22
Phosphorus (mg/kg dw)	2,957	3,243*	3,304*	2,766–3,105
Potassium (mg/kg dw)	3,752	3,960*	4,041*	3,367–4,188
Zinc (mg/kg dw)	20.5	19.1*	19.5*	20.4–25.1
β-carotene (mg/kg dw)	0.776	0.721*	0.749	1.14–1.85
Thiamine (mg/kg dw)	2.85	2.98*	2.96*	3.13–4.01
Niacin (mg/kg dw)	25.2	23.6*	23.6*	17.8–26.6
Pyridoxine (mg/kg dw)	5.15	4.56*	4.52*	4.80–7.03
Oleic acid (18:1) (% FA)	28.5	28.0	27.7*	22.0–27.2
Linoleic acid (18:2) (% FA)	53.4	53.9*	54.1*	54.9–61.4
Arachidic acid (20:0) (% FA)	0.430	0.424*	0.424*	0.377–0.408
Eicosenoic acid (20:1) (% FA)	0.249	0.240*	0.239*	0.252–0.309
Ferulic acid (mg/kg dw)	2,752	2,584*	2,627*	1,650–2,420
Inositol (μg/g dw)	2,425	2,603*	2,557	2,417–2,682
Phytic acid (% dw)	0.819	0.907*	0.890*	0.781–0.893

DW: dry weight; % FA: percentage of total fatty acids.
(a): Not treated with the target herbicides.
(b): Treated with the target herbicides.
(c): Additional information 28/7/2014/Appendix 16.

3.3.2.4. Conclusion

The EFSA GMO Panel concludes that none of the observed differences in the agronomic, phenotypic and compositional characteristics of grain and forage identified between maize Bt11 × 59122 × MIR604 × 1507 × GA21 and its conventional counterpart requires further assessment regarding food and feed safety.

The differences in agronomic and phenotypic characteristics are further assessed for their potential environmental impact in Section 3.3.4.

3.3.3. Food and feed safety assessment

3.3.3.1. Effect of processing[22]

Based on the outcome of the comparative assessment, processing of the five-event stack maize Bt11 × 59122 × MIR604 × 1507 × GA21 into food and feed products is not expected to result in products different from those of commercial non-GM maize varieties.

3.3.3.2. Toxicology

Toxicological assessment of newly expressed proteins

Eight proteins are newly expressed in the five-event stack maize (Section 3.3.1.3). The EFSA GMO Panel has previously assessed these proteins individually in the context of the single events, and no safety concerns were identified.

The three enzymatic proteins (PAT, PMI and mEPSPS) act on unrelated substrates and are not expected to interact. The five insecticidal proteins (Cry1Ab, Cry34Ab1 and Cry35Ab1, mCry3A, Cry1F) act through cellular receptors found in target insect species. It is reported that the gastrointestinal tract of mammals, including humans, lacks receptors with high specific affinity to Cry proteins (Hammond et al., 2013; Koch et al., 2015).

On the basis of the known biological function of the individual newly expressed proteins (Table 4), there is currently no expectation for possible interactions relevant to the food and feed safety assessment of the five-event stack maize Bt11 × 59122 × MIR604 × 1507 × GA21.

The EFSA GMO Panel concludes that there are no safety concerns to human and animal health related to the newly expressed proteins Cry1Ab, Cry34Ab1 and Cry35Ab1, mCry3A, Cry1F, PAT, PMI and mEPSPS in the five-event stack maize Bt11 × 59122 × MIR604 × 1507 × GA21.

Toxicological assessment of components other than newly expressed proteins

The five-event stack maize does not show any compositional difference to its conventional counterpart that would require further assessment (see Section 3.3.2.3).

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

None of the observed differences in the composition of the food/feed derived from maize Bt11 × 59122 × MIR604 × TC1507 × GA21 (see Section 3.3.2.3) require further assessment regarding food and feed safety. Therefore, according to EFSA (EFSA GMO Panel, 2011), no animal studies on the food/feed derived from maize Bt11 × 59122 × MIR604 × 1507 × GA21 are required. However, the applicant provided a broiler study which was considered by the EFSA GMO Panel.

A 50-day feeding study with a total of 540 (half male and half female) cross-line chickens for fattening (Ross 344-males × Ross 708-females) was performed.[23] The birds were randomly allocated to three dietary groups with 180 chicks per treatment (12 pens per treatment, 15 birds per pen, half for each sex[24]). Because of water supply problems two pens were excluded from the study. Birds were fed diets containing maize Bt11 × 59122 × MIR604 × 1507 × GA21 (identity verified by polymerase chain reaction (PCR)), containing near-isogenic maize or one non-GM commercial variety (NCSU 2009[25]). The starter (1–15 days), grower (16–34 days) and finisher GM diets consisted of 53.81%, 58.51% and 63.61% maize grain, respectively.[26] Before feed formulation, all maize varieties were analysed for proximates, amino acids and mycotoxins. The metabolisable energy was calculated for each maize grain source. All diets were balanced for metabolisable energy, crude protein and amino acids. Pelleted diets (starter crumbled) and water were offered *ad libitum*. The concentration of proteins Cry1Ab, Cry34Ab1, Cry35Ab1, mCry3A, PMI, Cry1F and mEPSPS were determined analytically in maize grain and the poultry diets prepared with this grain. Chickens were observed twice daily for clinical signs, injuries and mortalities; deaths were recorded. Mean body weights and feed intake were measured per pen at day 1, 16, 34 and 48. At the end of the study, two birds per pen were randomly selected and processed in order to determine carcass (meat) yield. Body weight, feed intake, feed conversion ratio and mortality data were statistically analysed using a two-way ANOVA (dietary

[22] Dossier: Part I – Section D7.6.
[23] Dossier: Part I – Section D7.8.4 and Appendix 28.
[24] In the final design, there were five pens and 75 birds per sex per transgenic treatment, due to a water pressure problem which resulted in the loss of two pens, each containing 15 birds, one pen per sex on this treatment.
[25] Commercially available, locally grown lot of North Carolina (NC, USA) maize grain from the 2009 season.
[26] Near-isogenic diets (54.20%, 58.47% and 62.93%) and non-GM commercial variety diets (53.49%, 56.95% and 62.12%).

group × sex), to determine statistical differences among groups fed diets prepared from the three maize grain sources, and between males and females. Carcass data were analysed for effects due to maize grain source within sex using a one-way ANOVA. Upon EFSA request, an additional statistical analysis was conducted, wherein a direct comparison was made between the Bt11 × 59122 × MIR604 × TC1507 × GA21 feeding group and the non-GM, near-isogenic control. Overall mortality was low (< 2%) with no significant difference between the groups.

No significant interactions (diet × sex) were detected for body weights, feed intake and feed conversion ratio at any time. No statistically significant differences in body weight between dietary groups were found at any time. No statistically significant differences were observed in feed intake of starter, grower nor finisher in broilers fed the GM diet and the non-GM diets. Considering feed intake in the entire period (0–48 days), lower consumption in broilers fed the GM diet was identified as compared to those fed non-GM diets. Higher moisture content in GM diet might contribute to this difference. There were no statistically significant differences in the carcass portions in both sexes considered relevant by the EFSA GMO Panel. All above mentioned endpoints obtained from the two groups (Bt11 × 59122 × MIR604 × TC1507 × GA21 transgenic treatment and the non-transgenic, near-isogenic control) were also subjected to the additional statistical analysis requested by EFSA which results were in agreement with previous ones.

The EFSA GMO Panel concluded that this study did not detect unintended effects, and showed that the five-event stack maize is as nutritious as its conventional counterpart and the non-GM commercial variety tested in this study.

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, as no single piece of information or experimental method yields sufficient evidence to predict allergenicity (EFSA, 2006a; 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[27]

For allergenicity, the EFSA GMO Panel has previously evaluated the safety of the Cry1Ab, mCry3A, Cry34Ab1, Cry35Ab1, Cry1F, 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 EFSA GMO Panel has become available. Based on the 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 five-event stack maize affecting allergenicity were identified.

For adjuvanticity, proteins derived from *B. 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 has previously evaluated the safety of the Cry1Ab, mCry3A, Cry34Ab1, Cry35Ab1 and Cry1F 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 this five-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 this five-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.

[27] Dossier: Part I – Section D7.9.1 and additional information: 3/5/2013, 30/7/2015, 17/12/2015 (spontaneous submission), 4/4/2016 (updated bioinformatics).

Assessment of allergenicity of GM plant products[28]

The EFSA GMO Panel regularly reviews the available publications on food allergy to maize (EFSA GMO Panel, 2013). However, to date, maize has not been considered to be a common allergenic food[29] (OECD, 2002). Therefore, the EFSA 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 and 3.3.2.3), the EFSA GMO Panel identified no indications of a potentially increased allergenicity of food and feed derived from the five-event stack maize with respect to that derived from its conventional counterpart.

3.3.3.5. Nutritional assessment of GM food/feed[30]

The intended traits of maize Bt11 × 59122 × MIR604 × 1507 × GA21 are herbicide tolerance and insecticide resistance, with no intention to alter the nutritional parameters. Comparison of the composition of the five-event stack maize Bt11 × 59122 × MIR604 × 1507 × GA21 with its conventional counterpart did not identify differences that would require a nutritional assessment as regards to food and feed (see Section 3.3.2.3). From these data, the nutritional characteristics of the five-event stack maize Bt11 × 59122 × MIR604 × 1507 × GA21 -derived food and feed are not expected to differ from those of conventional counterpart. This conclusion is also supported by the 50-day feeding study with chickens for fattening (Section 3.3.3.3).

3.3.3.6. Conclusion

The newly expressed proteins in the five-event stack maize do not raise safety concerns for human and animal health. No interactions between these proteins relevant for food and feed safety were identified. Similarly, the EFSA GMO Panel did not identify indications of safety concerns regarding allergenicity or adjuvanticity with the presence of newly expressed proteins in this five-event stack maize, or regarding the overall allergenicity of the five-event stack maize. The five-event stack maize is as nutritious as its conventional counterpart and the non-GM commercial variety used.

3.3.4. Environmental risk assessment[31]

The approach followed by the GMO Panel to assess environmental risks is to consider the scope of the five-event stack maize, the modes of action of the introduced traits, the possible interactions and the outcomes of the molecular characterization, as well as of the comparative analysis.

Considering the scope (which excludes cultivation) of the five-event stack maize, the environmental risk assessment (ERA) is mainly concerned with (i) exposure of bacteria to recombinant DNA in the gastrointestinal tract of animal fed GM material and bacteria present in environments exposed to faecal material; and (ii) accidental release into the environment of viable grains of the five-event stack maize during transportation and processing.

3.3.4.1. Persistence and invasiveness of the GM plant[32]

Maize is highly domesticated, not winter hardy in colder regions of Europe, and generally unable to survive in the environment without appropriate management. Occasional maize plants may occur outside cultivation areas but survival is limited mainly by a combination of low competitiveness, the absence of a dormancy phase and susceptibility to plant pathogens, herbivores and cold climate conditions (OECD, 2002). In fields, maize volunteers may arise under some environmental conditions (mild winters). Observations done in the field during harvesting indicate that grain may survive and overwinter in some regions, resulting in volunteers in subsequent crops. The occurrence of maize volunteers has been reported in Spain and other European regions (e.g. Gruber et al., 2008). However, maize volunteers have been shown to grow weakly and flower asynchronously with the maize crop (Palaudelmàs et al., 2009).

[28] Dossier: Part I – Section D7.9.2.
[29] 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.
[30] Dossier: Part I – Section D7.10.
[31] Dossier: Part I – Section D9.
[32] Dossier: Part I – Section D9.1 and D9.2.

As mentioned in Section 3.3.2.1, a field trials study was carried out in the USA in 2009 to assess the agronomic and phenotypic performance[33] of the five-event stack maize in comparison with its conventional counterpart. Significantly different values were observed for two characteristics of the five-event stack maize, i.e. initial and final stand count. In both cases, the five-event stack maize showed a lower number of plants per plot (see Section 3.3.2.2). Due to the lack of seed germination studies, it was not possible to assess if the observed differences were related to a different germination capacity of the starting materials. A reduction in early stand count was also identified in the agronomic and phenotypic field trials performed during the comparative assessment of two events of the five-event stack maize, namely maize 59122 and GA21 (EFSA, 2007b; EFSA GMO Panel, 2013). The differences observed suggested a lower fitness of the five-event stack maize than that of its conventional counterpart (i.e. underperforming compared to its conventional counterpart). As no statistically significant differences were observed for the other assessed agronomic and phenotypic characteristics which may affect fitness characteristics of the five-event stack maize, the EFSA GMO Panel considers that these differences do not induce a change in the persistence and invasiveness of the five-event stack maize compared to conventional maize.

In addition to the data presented by the applicant, the EFSA GMO Panel is not aware of any scientific report of increased spread, establishment and survival capacity of the five-event stack maize or maize with comparable properties.

Therefore, the EFSA GMO Panel concludes that it is unlikely that the five-event stack maize would differ from conventional maize varieties in its ability to survive until subsequent season under European environmental conditions, if there was accidental release of viable GM maize grains into the environment. The occurrence of GM maize plants in the environment will thus be limited.

3.3.4.2. Gene transfer[34]

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer of DNA or through vertical gene flow by cross-pollination from flowering plants arising from spilled grains.

Plant-to-microorganism gene transfer

The potential for horizontal gene transfer of the recombinant DNA of the five single events to bacteria was assessed in previous opinions (see EFSA scientific opinions listed in Table 2). No concern for an unlikely, but theoretically possible, horizontal gene transfer of the recombinant genes to bacteria in the gut of animal fed GM material or other receiving environments was identified. Synergistic effects of the recombinant genes in increasing the likelihood for horizontal gene transfer, for instance combinations of recombinogenic sequences, were not identified. Therefore, the EFSA GMO Panel concludes that, in the context of its scope, the unlikely, but theoretically possible, horizontal transfer of recombinant genes from this five-event stack maize to bacteria does not raise any environmental safety concern.

Plant-to-plant gene transfer

Considering the scope of the five-event stack maize and the biology of maize, a possible pathway to harm pertains to the potential of occasional feral GM maize plants originating from accidental spillage of imported grains to transfer recombinant DNA to sexually cross-compatible plants. As pointed out above (Section 3.3.4.1), occurrence of feral GM maize is expected to be limited.

The extent of cross-pollination to other maize varieties will mainly depend on the scale of accidental release during transportation and processing and on successful establishment and subsequent flowering of the GM maize plant. For maize, vertical gene transfer is limited to other *Zea* species. Populations of sexually compatible indigenous wild relatives of maize are not known in Europe (Eastham and Sweet, 2002; OECD, 2003), therefore vertical gene transfer is not considered to be an environmental issue in the EU.

The flowering of occasional feral GM maize plants originating from accidental release during transportation and processing is unlikely to lead to dispersal of significant amounts of GM maize pollen onto other maize plants. Field observations performed on maize volunteers after GM maize cultivation in Spain revealed that maize volunteers had a low vigour, rarely had cobs and produced pollen that cross-pollinated neighbouring plants only at low levels (Palaudelmàs et al., 2009). Thus, the likelihood

[33] Dossier: Part I – Section D7.1/Appendix 14/Additional information on 3/5/2013.
[34] Dossier: Part I – Section D9.3.

of cross-pollination between cultivated maize and the occasional feral maize plants resulting from grain spillage is considered extremely low.

In conclusion, the EFSA GMO Panel is of the opinion that the likelihood of spread of genes from this GM maize in Europe will not differ from that of conventional maize varieties, even in the case of treatment with the intended herbicides.

3.3.4.3. Interactions of the GM plant with target organisms[35]

Interactions might occur between different Cry proteins depending on the arthropod species tested (EcoΣtat, 2014; De Schrijver et al., 2015). Considering the scope of application EFSA-GMO-DE-2011-99, and the low level of exposure of the environment to this GM maize, interactions of the GM seeds or plants arising from spilled grains with target organisms are not considered a relevant issue by the EFSA GMO Panel, regardless of potential synergistic interactions that might occur between the different Cry proteins.

3.3.4.4. Interactions of the GM plant with non-target organisms[36]

The EFSA GMO Panel has previously assessed the Cry1Ab, Cry1F, Cry34Ab1, Cry35Ab1 and mCry3 proteins individually in the context of the single maize events, and no safety concern was identified for non-target organisms. As mentioned in Section 3.3.4.3, interactions between Cry proteins, leading to synergistic insecticidal effects, might occur in other susceptible non-target species. Considering that environmental exposure of non-target organisms to stored GM grains, spilled GM grains or GM plants arising from spilled GM grains is limited, potential exposure of non-target organisms sensitive to either combination of the Cry1Ab, Cry1F Cry34Ab1, Cry35Ab1 or mCry3 proteins is likely to be very low and of no biological relevance.

The EFSA GMO Panel evaluated whether the Cry1Ab, Cry1F, Cry34Ab1, Cry35Ab1 and mCry3 proteins might potentially affect non-target organisms by entering the environment through faecal material of animals fed the five-event stack maize. Cry proteins are degraded by enzymatic activity in the gastrointestinal tract, meaning that only a very low amount of these proteins would remain intact to pass out in faeces. This was demonstrated for Cry1Ab (Einspanier et al., 2004; Lutz et al., 2005, 2006; Wiedemann et al., 2006; Guertler et al., 2008; Paul et al., 2010). Further degradation of the protein in the manure and faeces would take place because of microbiological proteolytic activity. In addition, there will be further degradation of the Cry proteins in soil reducing the possibility for exposure of potentially sensitive non-target organisms. While proteins, including insecticidal Cry proteins, may bind to clay minerals and organic substances in soil, thereby reducing their availability to microorganisms for degradation, there are no indications of persistence and accumulation of these proteins from GM crops in soil (Gruber et al., 2012; Valldor et al., 2015). The EFSA GMO Panel is not aware of evidence of released Bt proteins causing significant negative effects on soil microorganisms.

Considering the scope of the five-event stack maize, it can be concluded that the exposure of potentially sensitive non-target organisms to the Cry1Ab, Cry1F, Cry34Ab1, Cry35Ab1 and mCry3 proteins expressed in the five-event stack maize is likely to be very low and of no biological relevance, regardless of potential synergistic interactions that might occur between the different Cry proteins.

3.3.4.5. Interactions with the abiotic environment and biogeochemical cycles[37]

Considering the scope of the five-event stack maize and the low level of exposure to the environment, potential interactions with the abiotic environment and biogeochemical cycles were not considered a relevant issue by the EFSA GMO Panel.

3.3.4.6. Conclusion

The EFSA GMO Panel concludes that it is unlikely that the five-event stack maize would differ from conventional maize varieties in its ability to survive until subsequent seasons under the European environmental conditions. Considering the scope of the GM maize, interactions with the biotic and abiotic environment were not considered to be a relevant issue. Risks associated with an unlikely but theoretically possible horizontal gene transfer of recombinant DNA from the five-event stack maize to bacteria have not been identified.

[35] Dossier: Part I – Section D9.4.
[36] Dossier: Part I – Section D9.5.
[37] Dossier: Part I – Section D9.8 and D9.10.

Therefore, considering the combined traits and their potential interactions, the outcome of the comparative analysis, the route of exposure and the exposure levels, the EFSA GMO Panel concluded that this five-event stack maize would not raise safety concerns in the event of accidental release of viable GM maize grains into the environment.

3.3.5. Conclusion on the five-event stack maize Bt11 × 59122 × MIR604 × 1507 × GA21

The combination of maize events Bt11, 59122, MIR604, 1507 and GA21 in the five-event stack maize does not raise issues relating to molecular, agronomic, phenotypic or compositional characteristics that would require further investigations.

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

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

Considering the introduced traits and the outcome of the comparative analysis, the routes of exposure and limited environmental exposure levels, the EFSA GMO Panel concludes that the five-event stack maize would not raise safety concerns in the event of accidental release of viable GM maize seeds into the environment.

3.4. Risk assessment of the subcombinations

All 20 subcombinations included in the scope of this application as listed in Table 1 were not previously assessed by the EFSA GMO Panel and no specific data were available for any of them.

The risk assessment of the 20 subcombinations covered by the scope followed a weight-of-evidence approach that takes as a starting point results of the assessment of the single events, the data generated for the five-event stack maize, and all the data available for subcombinations previously assessed (2009b, 2009e, 2010a, 2010b, 2010c, 2015a) but not included in the scope.

The EFSA GMO Panel assessed to what extent a combination of any of these events resulting in stacks with fewer than five events (see Table 1) could result in interactions manifesting at protein or trait expression level not observed in the five-event stack (e.g. because of masking). The potential for such interactions was addressed by investigating the known biological functions of the newly expressed proteins, and the new data submitted.

Integrity of the inserts was demonstrated in the five-event stack (see Section 3.3.1.2). The EFSA GMO Panel finds no reasons to expect the loss of integrity in any of the other subcombinations that would result from interactions between the events.

The Cry1Ab, Cry34Ab1, Cry35Ab1, mCry3A, Cry1F, PAT, PMI and mEPSPS proteins levels in the five-event stack maize were comparable to the corresponding levels in the single maize events and showed no major changes that could be the result of interactions (see Section 3.3.1.3).

No indication of interactions between the events based on biological functions of the newly expressed proteins that would raise a safety issue was identified in the five-event stack maize. In addition, the data on genetic stability and protein expression from the previously assessed five subcombinations (Table 2) do not show any evidence of interactions. Therefore, there is no indication to suggest that the presence of one protein may mask or enhance the effects of the others and there is no reason to expect interactions that would alter the expression levels of these proteins in the 20 subcombinations included in the scope of this application. However, at relatively high doses, the Bt proteins might act as adjuvants (see Section 3.3.3.4). Given the anticipated expression levels in the subcombinations that are similar to those measured in the singles, the five-event stack and the subcombinations already assessed (EFSA, 2005a, 2009b, 2009e, 2010a, 2010b; EFSA GMO Panel, 2015a), this scenario is considered extremely unlikely. However, to mitigate uncertainty, the EFSA GMO Panel has included a recommendation that, if any of the subcombinations were to be created via targeted breeding approaches and commercialised in the future, the applicant should provide relevant information including expression levels of the newly expressed proteins.

It is not expected that any combination of the newly expressed proteins would impact on the gross composition and consequently the nutritional characteristics of the maize variety into which they may be introduced. This was shown by the comparative analyses of the five-event stack maize and

confirmed by the comparative analyses of five subcombinations with their conventional counterparts (EFSA, 2004, 2005a, 2007b,c, 2009a, 2009d).

Considering the scope of the application, the mode of action of the introduced traits, the data available for various stacks and the poor ability of maize to survive outside cultivated land, the EFSA GMO Panel is of the opinion that different combinations of these events would not raise environmental concerns.

The 20 subcombinations included in the scope of this application are expected to be as safe as the five-event stack maize.

3.5. Post-market monitoring

3.5.1. Post-market monitoring of GM food/feed[38]

The EFSA GMO Panel considers that post-market monitoring of GM food/feed is not necessary, given the absence of safety concerns identified for the five-event stack maize and the 20 lower subcombinations. If these subcombinations were to be created via targeted breeding approaches and imported into the EU, the requirement for monitoring should be considered on the basis of new data provided.

3.5.2. Post-market environmental monitoring[39]

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 which were not anticipated in the ERA.

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

As the ERA did not identify potential adverse environmental effects due to the five-event stack maize (Section 3.3.4), no case-specific monitoring is required.

The PMEM plans proposed by the applicant for the five-event stack maize and for the 20 subcombinations covered by the scope of application EFSA-GMO-DE-2011-99 includes (1) the description of a monitoring approach involving operators (federations involved in maize import and processing), reporting to applicants, 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) the use of networks of existing surveillance systems (Lecoq et al., 2007; Windels et al., 2008). The applicant proposes to submit a PMEM report on an annual basis.

The EFSA GMO Panel is of the opinion that the PMEM plan proposed by the applicant is in line with the scope of the five-event stack maize. As no potential adverse environmental effects were identified, case-specific monitoring was not considered necessary. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plans.

4. Overall conclusions

No new data on the single maize events Bt11, 59122, MIR604, 1507 and GA21 that would lead to a modification of the original conclusions on their safety were identified. The combination of maize events Bt11, 59122, MIR604, 1507 and GA21 in the five-event stack maize did not give rise to issues – relating to molecular, agronomic/phenotypic or compositional characteristics – regarding food and feed safety.

The newly expressed proteins in the five-event stack maize did not raise concerns for human and animal health. The compositional data indicate that maize Bt11 × 59122 × MIR604 × 1507 × GA21 is expected to be as nutritious as its conventional counterpart. This was confirmed by the results of an animal feeding study in chickens for fattening.

The EFSA GMO Panel considers that there is no reason to expect interactions that could impact on food and feed safety. No safety concerns are foreseen for any subcombination of the individual events.

[38] Dossier: Part I – Section D7.11.
[39] Dossier: Part I – Section D9.11/Additional information: 4/4/2016.

Considering the introduced traits and the outcome of the comparative analysis, the routes of exposure and limited exposure levels, the EFSA GMO Panel concluded that the five-event stack maize would not raise safety concerns in case of accidental release of viable GM maize seeds into the environment, irrespective of possible interactions between the individual events within this five-event stack maize.

Moreover, in the light of the scope of the application, the data available for various subcombinations and the poor ability of maize to survive outside cultivated land, the EFSA GMO Panel is of the opinion that any subcombinations of the individual events would not raise environmental safety concerns. Post-market monitoring of food/feed derived from maize Bt11 × 59122 × MIR604 × 1507 × GA21 or the 20 subcombinations is not considered necessary. The EFSA GMO Panel considers the scope of the PMEM plan provided by the applicant is consistent with the scope of this application. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the PMEM plans.

The EFSA GMO Panel did not find indications that the subcombinations, resulting from combination of any of the single events included in the five-event stack maize, would raise safety concerns. However, no specific data were submitted for the subcombinations included in the scope of this application. For these subcombinations, the EFSA GMO Panel has drawn conclusions on a weight-of-evidence approach, which identified uncertainties due to the absence of specific data.

In order to reduce these uncertainties and to confirm assumptions made for the assessment of these subcombinations, the EFSA GMO Panel considers that the applicant should provide relevant information, if these subcombinations were to be created via targeted breeding approaches and imported into the EU in the future. In this case, this information should focus on expression levels of the newly expressed proteins.

A minority opinion expressed by an EFSA GMO Panel member is appended to this opinion.

Documentation provided to EFSA

1) Letter from the Competent Authority of Germany, received on 7 July 2011, concerning a request for placing on the market of genetically modified maize Bt11 × 59122 × MIR604 × 1507 × GA21 submitted by Syngenta Crop Protection AG in accordance with Regulation (EC) No 1829/2003 (application reference EFSA-GMO-DE-2011-99).
2) Acknowledgement letter dated 21 July 2009 from EFSA to the Competent Authority of Germany.
3) Letter from EFSA to applicant dated 17 August 2011 requesting additional information under completeness check.
4) Letter from EFSA to applicant dated 9 November 2011 requesting a consolidated version of the application and clarifications on the scope.
5) Letter from applicant to EFSA received on 12 December 2011 providing a timeline for submission of responses.
6) Letter from applicant to EFSA received on 21 December 2011 providing additional information under completeness check.
7) Letter from applicant to EFSA received on 21 December 2011 providing clarifications on the scope and a consolidated version of the application.
8) Letter from EFSA to applicant dated 25 January 2012 requesting additional information under completeness check.
9) Letter from applicant to EFSA received on 15 March 2012 requesting clarifications on the progress of six Syngenta applications.
10) Letter from applicant to EFSA received on 2 April 2012 providing additional information under completeness check.
11) Letter from EFSA to applicant dated 6 June 2012 providing clarifications requested on 15 March 2012.
12) Letter from EFSA to applicant dated 14 June 2012 delivering the 'Statement of Validity' of application for the placing on the market of genetically modified maize Bt11 × 59122 × MIR604 × 1507 × GA21 submitted by Syngenta Crop Protection AG in accordance with Regulation (EC) No 1829/2003.
13) Letter from EURL-GMFF to EFSA dated 20 June 2012 asking EFSA to stop the clock.
14) Letter from EFSA to applicant dated 22 June 2012 requesting additional information and stopping the clock on behalf of the EURL-GMFF.

15) Letter from EFSA to applicant dated 29 August 2012 re-starting the clock on behalf of the EURL-GMFF.
16) Letter from EFSA to applicant dated 7 December 2012 requesting additional information and stopping the clock.
17) Letter from applicant to EFSA received on 31 January 2013 providing a timeline for submission of responses.
18) Letter from EFSA to applicant dated 5 February 2013 requesting additional information and maintaining the clock stopped
19) Letter from EURL-GMFF to EFSA dated 27 March 2013 asking EFSA to stop the clock.
20) Letter from EFSA to applicant dated 2 April 2013 requesting additional information on behalf of the EURL-GMFF maintaining the clock stopped.
21) Letter from applicant to EFSA received on 3 May 2013 providing additional information.
22) Letter from EFSA to applicant dated 23 September 2013 requesting additional information and maintaining the clock stopped.
23) Letter from applicant to EFSA received on 4 November 2013 providing a timeline for submission of responses.
24) Letter from EURL-GMFF dated 4 March 2014 asking EFSA to re-start the clock on behalf of EURL-GMFF.
25) Letter from EFSA to applicant dated 6 March 2014 re-starting the clock on behalf of the EURL-GMFF and keeping it stopped for EFSA.
26) Letter from EFSA to applicant dated 12 March 2014 requesting additional information and maintaining the clock stopped.
27) Letter from applicant to EFSA received on 7 May 2014 providing a timeline for submission of responses.
28) Letter from applicant to EFSA received on 23 June 2014 providing additional information (the response includes info requested on 23/9/2013).
29) Letter from applicant to EFSA received on 28 July 2014 providing additional information spontaneously.
30) Letter from applicant to EFSA received on 30 September 2014 providing additional information spontaneously.
31) Letter from EFSA to applicant dated 27 October 2014 requesting additional information and maintaining the clock stopped.
32) Letter from applicant to EFSA received on 21 November 2014 providing additional information.
33) Letter from applicant to EFSA received on 28 November 2014 providing a timeline for submission of responses.
34) Letter from applicant to EFSA received on 20 January 2015 providing a timeline for submission of responses.
35) Letter from applicant to EFSA received on 27 May 2015 requesting clarifications on an EFSA request on Bioinformatics.
36) Letter from EFSA to applicant dated 18 June 2015 providing clarifications requested regarding Bioinformatics.
37) Letter from applicant to EFSA received on 21 July 2015 providing information regarding sequencing of Events MIR604 and GA21.
38) Letter from applicant to EFSA received on 27 July 2015 providing additional information.
39) Letter from EFSA to applicant dated 18 September 2015 requesting additional information and maintaining the clock stopped.
40) Letter from applicant to EFSA received on 24 September 2015 providing additional information.
41) Letter from EFSA to applicant dated 7 December 2015 re-starting the clock.
42) Letter from applicant to EFSA received on 17 December 2015 providing complementary information spontaneously.
43) Letter from EFSA to applicant dated 23 December 2015 requesting additional information and stopping the clock.
44) Letter from applicant to EFSA received on 8 February 2016 providing a timeline for submission of responses.
45) Letter from applicant to EFSA received on 4 April 2016 requesting a modification of the scope of the application.

46) Letter from applicant to EFSA received on 4 April 2016 providing additional information.
47) Communication from EFSA to applicant re-starting the clock on 4 April 2016.
48) Letter from applicant to the German Competent Authority and to EFSA received on 4 April 2016 regarding the change of scope of the application.

References

Chen E and Stacy C, 2003. Modified Cry3A toxins having increased toxicity to corn rootworm, their nucleic acid sequences, and methods for controlling plant pests. Patent Cooperation Treaty International Publication Number WO 03/018810 A2, 127 pp.

Codex Alimentarius, 2009. Foods derived from modern biotechnology. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme. Rome, Italy. 85 pp.

De Schrijver A, De Clercq P, de Maagd RA and van Frankenhuyzen K, 2015. Relevance of Bt toxin interaction studies for environmental risk assessment of genetically modified crops. Plant Biotechnology Journal, 13, 1221–1223.

Eastham K and Sweet J, 2002. Genetically modified organisms (GMOs): the significance of gene flow through pollen transfer. European Environment Agency. Available online: http://www.eea.europa.eu/publications/environmental_issue_report_2002_28

EcoΣtat, 2014. *Bacillus thuringiensis* toxins: their mode of action and the potential interaction between them.

EFSA (European Food Safety Authority), 2004. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the Notification (Reference C/NL/00/10) for the placing on the market of insect-tolerant genetically modified 1507 maize, for import and processing, under Part C of Directive 2001/18/EC from Pioneer Hi-Bred International/Mycogen Seeds. EFSA Journal 2004;2(10):124, 1–18. doi: 10.2903/j.efsa.2004.124

EFSA (European Food Safety Authority), 2005a. Opinion of the Panel on Genetically Modified Organisms (GMO Panel) on a request from the Commission related to the notification (Reference C/F/96/05.10) for the placing on the market of insect-tolerant genetically modified maize Bt11, for cultivation, feed and industrial processing, under Part C of Directive 2001/18/EC from Syngenta Seeds. EFSA Journal 2005;3(5):213, 1–33. doi: 10.2903/j.efsa.2005.213

EFSA (European Food Safety Authority), 2005b. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the notification (Reference C/ES/01/01) for the placing on the market of insect-tolerant genetically modified maize 1507 for import, feed and industrial processing and cultivation, under Part C of Directive 2001/18/EC from Pioneer Hi-Bred International/Mycogen Seeds. EFSA Journal 2005;3(3):181, 1–33. doi: 10.2903/j.efsa.2005.181

EFSA (European Food Safety Authority), 2006. Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. EFSA Journal 2006;4(4):99, 1–100. (Question No EFSA-Q-2003-005). doi: 10.2903/j.efsa.2006.99

EFSA (European Food Safety Authority), 2007a. Guidance document of the Panel on Genetically Modified Organisms (GMO Panel) on genetically modified organisms for the risk assessment of genetically modified plants containing stacked transformation events. EFSA Journal 2007;5(7):512, 1–5. doi: 10.2903/j.efsa.2007.512

EFSA (European Food Safety Authority), 2007b. Opinion of the Scientific Panel on genetically modified organisms [GMO]on an application (Reference EFSA-GMO-NL-2005-12) for the placing on the market of insect resistant genetically modified maize 59122, for food and feed uses, import and processing under Regulation (EC) No 1829/2003, from Pioneer Hi-Bred International, Inc. and Mycogen Seeds, c/o Dow Agrosciences LLC (Reference EFSA-Q-2005-045). EFSA Journal 2007;5(4):470, 1–25. doi: 10.2903/j.efsa.2007.470

EFSA (European Food Safety Authority), 2007c. Opinion of the Panel on Genetically Modified Organisms (GMO Panel) on application (Reference EFSA-GMO-UK-2005-19 and EFSA-GMO-RX-GA21) for the placing on the market of glyphosate-tolerant genetically modified maize GA21, for food and feed uses, import and processing and for renewal of the authorisation of maize GA21 as existing product, both under Regulation (EC) No 1829/2003 from Syngenta Seeds S.A.S. on behalf of Syngenta Crop Protection AG. EFSA Journal 2007;5(10):541, 1–25. doi: 10.2903/j.efsa.2007.541

EFSA (European Food Safety Authority), 2009a. Opinion of the Panel on Genetically Modified Organisms (GMO Panel) on application reference EFSA-GMO-RX-Bt11 for renewal of the authorisation of existing products produced from insect-resistant genetically modified maize Bt11, under Regulation (EC) No 1829/2003 from Syngenta. EFSA Journal 2009;7(2):977, 1–13. doi: 10.2903/j.efsa.2009.977

EFSA (European Food Safety Authority), 2009b. Scientific Opinion of the Panel on Genetically Modified Organisms (GMO Panel) on application (Reference EFSA-GMO-UK-2005-11) for the placing on the market of insect-resistant genetically modified maize MIR604 event, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Syngenta Seeds S.A.S on behalf of Syngenta Crop Protection AG. EFSA Journal 2009;7(7):1193, 1–26. doi: 10.2903/j.efsa.2009.1193

EFSA (European Food Safety Authority), 2009c. Scientific Opinion of the Panel on Genetically Modified Organisms on Application (EFSA-GMO-RX-1507) for renewal of authorisation for the continued marketing of existing products produced from maize 1507 for feed use, under Regulation (EC) No 1829/2003 from Pioneer Hi-Bred International, Inc./Mycogen Seeds. EFSA Journal 2009;7(6):1138, 1–11. doi: 10.2903/j.efsa.2009.1138

EFSA (European Food Safety Authority), 2009d. Opinion of the Panel on Genetically Modified Organisms (GMO Panel) on application (Reference EFSA-GMO-UK-2005-11) for the placing on the market of insect-resistant genetically modified maize MIR604 event, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Syngenta Seeds S.A.S on behalf of Syngenta Crop Protection AG. EFSA Journal 2009; 1193, 26 pp. doi:10.2903/j.efsa.2009.1193

EFSA (European Food Safety Authority), 2010a. Opinion of the Scientific Panel on Genetically Modified Organisms (GMO Panel) on application (Reference EFSA-GMO-UK-2007-48) for the placing on the market of insect resistant and herbicide tolerant genetically modified maize MIR604 × GA21 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Syngenta Seeds. EFSA Journal 2010;8(5):1611, 30 pp. doi:10.2903/j.efsa.2010.1611

EFSA (European Food Safety Authority), 2010b. Opinion of the Scientific Panel on Genetically Modified Organisms (GMO Panel) on application (Reference EFSA-GMO-UK-2007-50) for the placing on the market of insect resistant and herbicide tolerant genetically modified maize Bt11 × MIR604 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Syngenta Seeds. EFSA Journal 2010;8(5):1614, 30 pp. doi:10.2903/j.efsa.2010.1614

EFSA (European Food Safety Authority), 2010c. Opinion of the Scientific Panel on Genetically Modified Organisms (GMO Panel)on application (Reference EFSA-GMO-UK-2008-56) for the placing on the market of insect resistant and herbicide tolerant genetically modified maize Bt11 × MIR604 × GA21, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Syngenta Seeds. EFSA Journal 2010;8(5):1616, 30 pp. doi:10.2903/j.efsa.2010.1616

EFSA (European Food Safety Authority), 2011a. Guidance on the post-market environmental monitoring (PMEM) of genetically modified plants. EFSA Journal 2011;9(8):1–40, 2316 pp. doi: 10.2903/j.efsa.2011.2316. Available online: http://www.efsa.europa.eu/en/efsajournal/doc/2316.pdf

EFSA (European Food Safety Authority), 2011b. Opinion of the Panel on Genetically Modified Organisms (GMO Panel) on application (EFSA-GMO-UK-2008-60) for placing on the market of genetically modified herbicide tolerant maize GA21 for food and feed uses, import, processing and cultivation under Regulation (EC) No 1829/2003 from Syngenta Seeds. EFSA Journal 2011;9(12):2480, 94 pp. doi:10.2903/j.efsa.2011.2480

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010. Guidance on the environmental risk assessment of genetically modified plants. EFSA Journal 2010;8(11):1879, 111 pp. doi:10.2903/j.efsa.2010.1879

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2011. Guidance for risk assessment of food and feed from genetically modified plants. EFSA Journal 2011;9(5):2150, 37 pp. doi:10.2903/j.efsa.2011.2150

EFSA GMO Panel (Panel on Genetically Modified Organisms), 2012b. Scientific Opinion updating the risk assessment conclusions and risk management recommendations on the genetically modified insect resistant maize Bt11. EFSA Journal 2012;10(12):3018, 104 pp. doi:10.2903/j.efsa.2012.3018

EFSA GMO Panel (Panel on Genetically Modified Organisms), 2013. Scientific Opinion on an application from Pioneer Hi-Bred International and Dow AgroSciences LLC (EFSA-GMO-NL-2005-23) for placing on the market of genetically modified maize 59122 for food and feed uses, import, processing and cultivation under Regulation (EC) No 1829/2003. EFSA Journal 2013;11(3):3135, 104 pp. doi:10.2903/j.efsa.2013.3135

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2015a. Scientific Opinion on an application by Syngenta (EFSA-GMO-DE-2009-66) for placing on the market of herbicide tolerant and insect resistant maize Bt11 × MIR162 × MIR604 × GA21 and subcombinations independently of their origin for food and feed uses, import and processing under Regulation (EC) No 1829/2003. EFSA Journal 2015;13(12):4297, 34 pp. doi:10.2903/j.efsa.2015.4297

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2015b. Statement on the risk assessment of new sequencing data on GM maize event MIR604. EFSA Journal 2015;13(10):4255, 6 pp. doi:10.2903/j.efsa.2015.4255

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2015c. Statement on the risk assessment of new sequencing data on GM maize event GA21. EFSA Journal 2015;13(11):4296. doi: 10.2903/j.efsa.2015.4296

Einspanier R, Lutz B, Rief S, Berezina O, Zverlov V, Schwarz W and Mayer J, 2004. Tracing residual recombinant feed molecules during digestion and rumen bacterial diversity in cattle fed transgenic maize. European Food Research and Technology, 218, 269–273.

Ellis RT, Stockhoff BA, Stamp L, Schnepf HE and Schwab GE, 2002. Novel *Bacillus thuringiensis* binary insecticidal crystal proteins active on western corn rootworm, *Diabrotica virgifera virgifera* LeConte. Applied and Environmental Microbiology, 68, 1137–1145.

Gruber S, Colbach N, Barbottin A and Pekrun C, 2008. Post-harvest gene escape and approaches for minimizing it. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 3, 1–17.

Gruber H, Paul V and Meyer HHD, 2012. Determination of insecticidal Cry1Ab protein in soil collected in the final growing seasons of a nine-year field trial of Bt-maize MON810. Transgenic Research, 21, 77–88. doi: 10.1007/s11248-011-9509-7

Guertler P, Lutz B, Kuehn R, Meyer HHD, Einspanier R, Killermann B and Albrecht C, 2008. Fate of recombinant DNA and Cry1Ab protein after ingestion and dispersal of genetically modified maize in comparison to rapeseed by fallow deer (*Dama dama*). European Journal of Wildlife Research, 54, 36–43.

Hammond B, Kough J, Herouet-Guicheney C and Jez JM; on behalf of the ILSI International Food Biotechnology Committee Task Force on the Use of Mammalian Toxicology Studies in the Safety Assessment of GM Foods, 2013. Toxicological evaluation of proteins introduced into food crops. Critical Reviews in Toxicology, 43(Suppl. 2), 25–42.

Herrmann KM, 1995. The Shikimate Pathway: early steps in the biosynthesis of aromatic compounds. Plant Cell, 7, 907–919.

Koch MS, Ward JM, Levine SL, Baum JA, Vicini JL and Hammond BG, 2015. The food and environmental safety of *Bt* crops. Frontiers in Plant Science, 6, 283. Published online 2015 April 29. doi: 10.3389/fpls.2015.00283

Lebrun M, Sailland A, Freyssinet G and Degryse E, 2003. Mutated 5-enoylpyruvylshikimate-3- phosphate synthase, gene coding for said protein and transformed plants containing said gene. Bayer CropScience S.A. (Lyons, FR) Patent No 6,566,587.

Lecoq E, Holt K, Janssens J, Legris G, Pleysier A, Tinland B and Wandelt C, 2007. General surveillance: roles and responsibilities the industry view. Journal of Consumer Protection and Food Safety, 2(S1), 25–28.

Lutz B, Wiedemann S, Einspanier R, Mayer J and Albrecht C, 2005. Degradation of Cry1Ab protein from genetically modified maize in the bovine gastrointestinal tract. Journal of Agricultural and Food Chemistry, 53, 1453–1456.

Lutz B, Wiedemann S and Albrecht C, 2006. Degradation of transgenic Cry1Ab DNA and protein in Bt-176 maize during the ensiling process. Journal of Animal Physiology and Animal Nutrition (Berlin), 90, 116–123.

Markovitz A, Sydiskis RJ and Lieberman MM, 1967. Genetic and biochemical studies on mannosenegative mutants that are deficient in phosphomannose isomerase in *Escherichia coli* K-12. Journal of Bacteriology, 94, 1492–1496.

Negrotto D, Jolley M, Beer S, Wenck AR and Hansen G, 2000. The use of phosphomannoseisomerase as a selectable marker to recover transgenic maize plants (*Zea mays* L.) via Agrobacterium transformation. Plant Cell Reports, 19, 798–803.

OECD (Organisation for Economic Co-operation and Development), 2002. Consensus Document oncompositional considerations for new varieties of maize (*Zea mays*): key food and feed nutrients, anti-nutrients and secondary plant metabolites. Series on the Safety of Novel Food and Feeds, No 6, 1–42.

OECD (Organisation for Economic Co-operation and Development), 2003. Consensus Document on the biology of Zea mays subsp. Mays (Maize). Series on Harmonisation of Regulatory Oversight in Biotechnology (ENV/JM/MONO(2003)11), No 27, 1–49.

Palaudelmàs M, Peñas G, Melé E, Serra J, Salvia J, Pla M, Nadal A and Messeguer J, 2009. Effect of volunteers on maize gene flow. Transgenic Research, 18, 583–594.

Paul V, Guertler P, Wiedemann S and Meyer HHD, 2010. Degradation of Cry1Ab protein from genetically modified maize (MON810) in relation to total dietary feed proteins in dairy cow digestion. Transgenic Research, 19, 683–689.

Schnepf E, Crickmore N, Van Rie J, Lereclus D, Baum J, Feitelson J, Zeigler DR and Dean DH, 1998. Bacillus thuringiensis and its pesticidal crystal proteins. Microbiology and Molecular Biology Reviews, 62, 775–806.

Sekar V, Thompson DV, Maroney MJ, Bookland RG and Adang MJ, 1987. Molecular cloning and characterization of the insecticidal crystal protein gene of *Bacillus thuringiensis* var. *tenebrionis*. Proceedings of the National Academy of Sciences of the United States of America, 84, 7036–7040.

Valldor P, Miethling-Graff R, Martens R and Tebbe CC, 2015. Fate of the insecticidal Cry1Ab protein of GM crops in two agricultural soils as revealed by 14C-tracer studies. Applied Microbiology and Biotechnology, 99, 7333–7341.

Vázquez RI, Moreno-Fierros L, Neri-Bazán L, de la Riva GA and López-Revilla R, 1999. *Bacillus thuringiensis* Cry1Ac protoxin is a potent systemic and mucosal adjuvant. Scandinavian Journal of Immunology, 49, 578–584.

Wiedemann S, Lutz B, Kurtz H, Schwarz FJ and Albrecht C, 2006. *In situ* studies on the time-dependent degradation of recombinant corn DNA and protein in the bovine rumen. Journal of Animal Science, 84, 135–144.

Windels P, Alcalde E, Lecoq E, Legris G, Pleysier A, Tinland B and Wandelt C, 2008. General surveillance for import and processing: the EuropaBio approach. Journal of Consumer Protection and Food Safety, 3(S2), 14–16.

Wohlleben W, Arnold W, Broer I, Hillemann D, Strauch E and Pühler A, 1988. Nucleotide sequence of the phosphinothricin N-acetyltransferase gene from *Streptomyces viridochromogenes* Tü494 and its expression in *Nicotiana tabacum*. Gene, 70, 25–37.

Abbreviations

ADF	acid detergent fibre
ANOVA	analysis of variance
Bt	*Bacillus thuringiensis*
CaMV	cauliflower mosaic virus
Cry	crystal protein

EcoΣtat	statistical consultancy in ecology, ecotoxicology and agriculture research
EFSA GMO Panel	Panel on Genetically Modified Organisms of the European Food Safety Authority
ELISA	enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	environmental risk assessment
EURL-GMFF	European Union Reference Laboratory for GM Food & Feed
FA	fatty acid
GM	genetically modified
GMO	genetically modified organism
IgE	immunoglobulin E
LOQ	limit of quantification
NDF	neutral detergent fibre
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PAT	phosphinothricin acetyltransferase
PCR	polymerase chain reaction
PMEM	post-market environmental monitoring
PMI	phosphomannose isomerase
TDF	total dietary fibre
UTR	untranslated region

Appendix A – Minority opinion

Application EFSA-GMO-DE-2011-99

(Bt11 × 59122 × MIR604 × 1507 × GA21 maize)

Minority Opinion

J.M. Wal, Member of the EFSA GMO Panel
Rapporteur of AP 99 for Food/Feed safety assessment

Summary

Application (AP) 99 includes the five-event stack Bt11 × 59122 × MIR604 × 1507 × GA21 maize and all sub-combinations that may derive from this stack independently of their origin. It is, however, noteworthy that six sub-combinations are out of the scope of the AP by decision of the Applicant, but the 20 other ones are still in the scope. This means that, according to the EU regulation, the adoption of the five-event stack Bt11 × 59122 × MIR604 × 1507 × GA21 maize will, automatically and simultaneously, result in the adoption of these 20 subcombinations.

This will apply if they are present by natural segregation during the cultivation of the authorized five-event stack or if they are produced on their own in the future by targeted conventional breeding techniques, using parental 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 20 sub-combinations have been provided by the Applicant, who also did not give a satisfactory rationale explaining the reasons why those data are missing and/or why he would consider that they are not necessary for the risk assessment.

This is a most important reason for expression of this minority opinion, considering that there cannot be two kinds of risk assessment, a comprehensive one based on a complete set of data and another one for which no specific data at all are available and which is based on assumptions and indirect considerations deduced by the Panel by the so called "weight of evidence approach" and extrapolation of data obtained for the single events, the five-event stack and other stacks that were submitted and assessed in other applications. In addition to this matter of principle, in the present case, this may result in uncontrolled risk for the health of human consumers in certain segments of the population.

Presentation of AP 99

The intended uses of this five-event stack are to control lepidopteran and coleopteran maize pests and provide tolerance to herbicides containing glufosinate ammonium or glyphosate.

The single events:

Bt11 (truncated Cry1Ab protein, PAT protein); **59122** (Cry34Ab1 protein+Cry35Ab1 protein, PAT protein); **MIR604** (mCry3A protein, PMI (Phospho Mannose Isomerase)); **1507** (Cry1F protein, PAT protein); **GA21** (mEPSPS protein), were assessed by EFSA.

In addition, 6 sub-combinations, i.e.:

MIR604 x GA21 (AP 48); Bt11 x GA21 (AP 49); Bt11 x MIR604 (AP 50); 1507 × 59122 (AP 15); Bt11 x MIR604 x GA21 (AP 56) and Bt11 × 1507 × 59122 (AP 15), were also assessed by EFSA in previous applications but they have been removed from the scope of AP 99 by the Applicant and are thus not directly concerned by this risk assessment.

Then 20 "new" sub-combinations that have never been assessed before by EFSA and that may have even not been produced yet, at least for some of them, are parts of AP 99 and of this risk assessment. Their list is the following:

Bt11 × MIR604 × 1507 × GA21; Bt11 × 59122 × MIR604 × GA21; Bt11 × 59122 × 1507 × GA21; 59122 × MIR604 × 1507 × GA21; Bt11 × 59122 × MIR604 × 1507;
Bt11 × 59122 × MIR604; Bt11 × 59122 × GA21; Bt11 × MIR604 × 1507; 59122 × MIR604 × 1507; 59122 × MIR604 × GA21; 59122 × 1507 × GA21; Bt11 × 1507 × GA21; MIR604 × 1507 × GA21; 59122 × MIR604 x GA21
Bt11 × 59122; Bt11 × 1507; 59122 × MIR604; 59122 × GA21; MIR604 × 1507; 1507 × GA21

No specific data has been provided for any of these 20 sub-combinations. In a response to the Commission (1), the Applicant has presented a compilation of general considerations of different nature to claim that those missing data were not necessary for the risk assessment.

This list cannot be considered a consistent rationale. Moreover some arguments provided appear to be contradictory.

It is thus mentioned in point ii)

"the Bt11 × 59122 × MIR604 × 1507 × GA21 maize ***and all of its sub-combinations independently of their origin have been produced by conventional breeding crosses*** of the GM maize single events Bt11, 59122, MIR604; 1507 and GA21 maize."

and in point x): ***"The analysis of the protein expression level also confirms*** that the crossing of the GM maize single events Bt11, 59122, MIR604; 1507 and GA21 results in no interaction between them in Bt11 × 59122 × MIR604 × 1507 × GA21 maize ***or the sub-combinations of fewer of these events independently of their origin."***

In such case, if those data have been produced as stated by the Applicant, they should have been provided.

Food/Feed safety assessment of AP 99

Comparative analyses

The Food/Feed Safety Assessment of the five-event stack has been performed according to the EFSA Guidance Documents, 2006 and 2007 (2, 3). During the comparative analysis with the conventional counterpart, a few differences were identified in the agronomic and phenotypic characteristics and in the composition of grain and forage. They were not considered relevant for Food and Feed safety and no further assessment was required.

As previously indicated, no specific data were provided for any of the 20 sub-combinations in the scope of AP99.

Toxicological assessment of the newly expressed proteins.

EFSA has previously performed a toxicological assessment of the newly expressed proteins (NEPs) individually in the context of the single event applications and no safety concerns were identified. The 3 insecticidal Bt proteins (i.e. produced by Bacillus thuringiensis), namely Cry1Ab, Cry1F, mCry3A, the herbicide tolerant proteins, i.e. PAT, mEPSPS and the marker protein PMI are rapidly degraded by pepsin in the conditions of the pepsin resistance test and their sequences show no significant homology to known protein toxins and allergens. They were therefore considered unlikely to present a health risk to humans or animals and no further assessment, including a 28-day repeated dose toxicity study, was required in the context of these applications.

However, a 14-day repeated dose toxicity study with the PAT protein was also performed and provided by the Applicant in the context of the application on 59122 maize. It confirmed the absence of adverse effects.

The two Bt proteins Cry34Ab1 and Cry35Ab1 expressed in 59122 maize structurally differ from Cry1Ab, Cry1F and Cry3A; they belong to the family of binary toxins and act synergistically. Therefore they are used and expressed together in GM crops. Because of such characteristics, a 28-day toxicity study was required by EFSA and it was performed by the Applicant using the mixture at a ratio Cry34Ab1/Cry35Ab1 of ca. 25 (e.g. same as that expressed in the plant). No treatment-related effects were observed.

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 and particularly humans because of their specific mode of action as insecticide (i.e. binding to specific receptors of insect gut mucosa with high affinity). 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 described in mice after high dose administrations of Cry1Ac by the intra gastric, intra peritoneal and intra nasal routes.

Among other effects, an antibody response against an unrelated protein has been observed (for review see 4). 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 doseresponse relationship and the manifestation of such an adjuvant activity in other Bt proteins than Cry1Ab and Cry1Ac. This issue has been extensively discussed when assessing the single events and it has then been shown by different research groups: i) that at the dose at which it is expressed in MON 810, Cry1Ab did not exert an allergenic or an adjuvant activity (at least for the MON 810 cultivars that have been tested) and ii) that the adjuvant activity would anyway differ from that of cholera toxin by its mode of action, which was

at this time a major concern also expressed by some Member State Competent Authorities (for review see 5). It was then concluded that in those single events the risk of adverse effects because of an adjuvant activity was unlikely.

In the present five-event stack the levels of expression of the NEPs are similar to those measured in the singles. The small increase observed in the concentration of the PAT protein does not raise concerns for human and animal health given the outcomes of the repeated dose toxicological study. In the case of Bt proteins, interactions have been described for their insecticidal activity but nothing is known regarding possible interactions or particularly additive effects in their mode of action as adjuvant in mammals. It differs from the mechanism of toxicity on insects and is now thought to be due to a dose related effect on the innate immunity system. However given the data on the low levels of expression of the NEPs in the five-event stack, it may be assumed from the literature that the manifestation of an adjuvant activity by the combination of the 3 newly expressed Bt proteins is unlikely even in the case of a possible additive effect. This conclusion also pertains to the sub-combinations derived from the five-event stack by natural segregation during its cultivation.

There is thus no disagreement with the opinion adopted by the GMO Panel regarding the fiveevent stack and the sub-combinations derived from this stack by natural segregation. The disagreement derives from the conclusion on the 20 "sub-combinations independently of their origin" for which no data have been provided. Some of these sub-combinations may already exist but they could mostly be produced in the future by targeted conventional breeding with parental lines different from those used in the 5 event stacks, the single events and sub-combinations previously assessed. Those new stacks would be de facto authorized and could be imported in the EU since in its opinion the EFSA GMO Panel concludes on the absence of safety concerns for the five-event stack maize and the twenty lower subcombinations and does not require any further assessment.

No clear reservation which might question this assumption is made regarding a possible higher expression level of the newly expressed Bt proteins compared to those actually measured in the 5 event stack, singles and other fully assessed applications.

However compositional data and actual concentrations of the NEPs are crucial to detect possible unintended effect and achieve a grounded 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 (6) and therefore it may cause an important variability in Bt protein concentrations which might impact on the safety. The risk of increased expression of the newly expressed Bt proteins in some of the "future" sub-combinations 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 99 including the 20 sub-combinations in question is for import and processing which suggests a limited exposure for consumers in the EU.

Nevertheless, should those sub-combinations (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 × 59122 × MIR604 × 1507 × GA21 maize.

In its opinion the GMO Panel states that it expects no adverse effect on human health; this expectation or assumption is based on so called "weight of evidence approach" and extrapolation of the data available for the singles, the five-event stack and some subcombinations, i.e. those already assessed in previous applications that are out of the scope of AP 99. However what kind of extrapolation is being made is not precisely defined. The criteria, procedure and the level of confidence that should be required for this extrapolation are not given and there is no critical appraisal of its limitations. No evaluation of the resulting uncertainty has been performed, e.g. using a probabilistic analysis, as recommended by the Draft Guidance on Uncertainty in EFSA Scientific Assessment (Revised for Internal Testing) of the EFSA Scientific Committee (7). These weaknesses may invalidate the general conclusion.

In addition one might consider that the role and remit of EFSA experts should be limited to check the validity and relevance of the data provided and the reliability of the outcomes of the safety assessment performed by the Applicant and not to develop arguments that could replace the missing data instead of the Applicant.

Conclusion

It is not acceptable that the same weight and reliability is 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 (i.e. the five-event stack and the whole package of 20 sub-combinations respectively in the present AP 99). In this case, the safety assessment of the 20 sub-combinations by the GMO Panel is based on assumptions that are not fully clarified and justified

and on so called weight of evidence approach and extrapolation which principle and limitations are also not described and evaluated in terms of uncertainty. This is not in accordance with the above-mentioned EFSA Guidance on Uncertainty.

The GMO Panel 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 20 subcombinations should they be produced and imported to the EU market in the future.

Consequently the message delivered in this opinion is unclear since the EFSA GMO Panel concludes that there is no safety concern (and therefore that a post-market monitoring programme of GM food/feed is not necessary) for any of the 20 sub combinations despite of the data gap but at the same time it "considers" that because of the uncertainty resulting from this data gap additional data should be provided if those "safe" sub-combinations were to be produced in the future.

In addition, mixing considerations that are the remit and responsibility of risk assessment or of risk management also adds to the confusion. Indeed recommendations might be a message for risk managers but it is not sufficient and the practical impact and regulatory value are unclear. It cannot substitute a comprehensive quantitative uncertainty analysis.

This is not only a question of principle since in the present case a risk for the human health may result from a possible over expression of NEPs if those sub-combinations were to be produced in other genetic backgrounds than the five-event stack. 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; therefore, it is not up to the EFSA GMO Panel Experts to a priori disregard potential unintended adverse effects and take responsibility for an incompletely assessed risk. In the future, a better knowledge of the mechanisms of action of NEPs (particularly of the different Bt proteins) and of the dose-(side) effect relationship would also allow decreasing the uncertainty of the risk assessment. Allergic reactions in general and consequently 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 and cultivated plant species. It would certainly be a shame if (re)questioning a possible role of GM crops would thus be triggered by this case.

Those are the arguments and serious concerns that justify this minority opinion which should be part of the risk assessment of AP 99. As stated in the Guidance on Uncertainty in EFSA Scientific Assessment (7):

"Experts often have differing views on the same question. ..... Where differences remain this is part of scientific uncertainty and should be reflected in the assessment report, either within the uncertainty analysis or, when appropriate, through EFSA's procedure for minority opinions, so it can be taken into account by decision-makers".

References

1. Letter of the Applicant to the Commission regarding the scope of Application EFSA-GMODE-2011-99 (Bt11 × 59122 × MIR604 × 1507 × GA21 maize) dated 21 February 2014, Annex A.

2. EFSA, 2006. Guidance document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed. EFSA Journal, 99, 1-100.

3. EFSA, 2007. Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events. EFSA Journal, 512, 1-5.

4. N. Rubio-Infante and L. Moreno-Fierros An overview of the safety and biological effects of Bacillus thuringiensis Cry toxins in mammals. Journal of Applied Toxicology, 2015, on line publication, (wileyonlinelibrary.com) DOI 10.1002/jat.3252

5. S S. Joshi, B. Barnett, N G. Doerrer, K Glenn, R A. Herman, C. Herouet-Guicheney, P. Hunst, J. Kough, G S. Ladics, S. McClain, S. Papineni, L K. Poulsen, JB. Rascle, AL. Tao, R. van Ree, J. Ward, C. C. Bowman. Assessment of potential adjuvanticity of Cry proteins Accepted Manuscript in Regulatory Toxicology and Pharmacology PII: S0273-2300(16)30094-0; DOI: 10.1016/j.yrtph.2016.04.005

6. J.J. Adamczyk, Jr. and W.R. Meredith, Jr. Genetic Basis for Variability of Cry1Ac Expression Among Commercial Transgenic *Bacillus thuringiensis* (Bt) Cotton Cultivars in the United States *The Journal of Cotton Science 8:17–23 (2004)*, Breeding and Genetics

7. EFSA, 2016. Draft Guidance on Uncertainty in EFSA Scientific Assessment (Revised for Internal Testing) of the EFSA Scientific Committee. March 2016.