TECHNICAL REPORT

APPROVED: 29 October 2015 PUBLISHED: 7 December 2015

Overall opinion of the European Food Safety Authority in accordance with Articles 6 and 18 of Regulation (EC) No 1829/2003 on application by Syngenta (reference EFSA-GMO-DE-2009-66) for the placing on the market of 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

European Food Safety Authority

Summary

This document provides an overall opinion of the European Food Safety Authority on genetically modified (GM) maize Bt11 × MIR162 × MIR604 × GA21 and subcombinations independently of their origin in accordance with the requirements of Articles 6 and 18 of Regulation (EC) No 1829/2003.

Following the submission of application EFSA-GMO-DE-2009-66 under Regulation (EC) No 1829/2003 from Syngenta, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) was asked to deliver a scientific opinion on the safety of herbicide tolerant genetically modified (GM) maize Bt11 × MIR162 × MIR604 × GA21 (hereafter referred to as 'four-event stack maize') and on all the ten subcombinations[1] (referred to as '*subcombinations independently of their origin*' in the Commission implementing regulation (EU) No 503/2013 [EC, 2013]). The scope defined by the applicant at the time of submission was "*all food and feed products containing, consisting or produced from Bt11 × MIR162 × MIR604 × GA21 maize including products from inbreds and hybrids obtained by conventional breeding of this stacked maize product. The application also covers the import and industrial processing of Bt11 × MIR162 × MIR604 × GA21 maize for all potential uses as any other maize*"[2].

In delivering its scientific opinion, the EFSA GMO Panel considered the data available on the four-event stack maize and the subcombinations, the scientific comments submitted by the Member States and the relevant scientific publications. The Scientific Panel on Genetically Modified Organisms (EFSA GMO Panel) has carried out the scientific assessment of GM maize Bt11 × MIR162 × MIR604 × GA21 and subcombinations independently of their origin in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. The EFSA GMO Panel previously assessed the four single events combined to produce a four-event stack maize Bt11 × MIR162 × MIR604 × GA21 and did not identify safety concerns. In this opinion, the EFSA GMO Panel assesses the four-event stack maize and all its subcombinations independently of their origin. No new data on the single events, leading to modification of the original conclusions on their safety, were identified. The molecular, agronomic, phenotypic and compositional data on the four-event stack maize did not give rise to safety concerns

[1] The 10 subcombinations are three-event stacks Bt11 × MIR162 × MIR604, Bt11 × MIR162 × GA21, Bt11 × MIR604 × GA21, MIR162 × MIR604 × GA21; and two-event stacks Bt11 × MIR162, Bt11 × MIR604, Bt11 × GA21, MIR162 × MIR604, MIR162 × GA21, MIR604 × GA21.

[2] After clarifications (letters received 14 June 2010, 15 September 2010, 15 March 2012, 6 June 2012, 8 July 2013 and 24 July 2013), the applicant notified EFSA that the scope of EFSA-GMO-DE-2009-66 was to "*include Bt11 x MIR 162 x MIR604 x GA21 maize and all subcombinations from Bt11 x MIR 162 x MIR604 x GA21 maize independently of their origin.*".

and there is no reason to expect interactions between the single events impacting on the food and feed safety of the four-event stack maize. Considering the routes of exposure and limited exposure levels, the Panel concludes that this four-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 four-event stack maize is as safe and as nutritious as its conventional counterpart in the context of its scope. Among the 10 subcombinations, four have been assessed previously and no safety concerns were identified. For the remaining six subcombinations, the EFSA GMO Panel followed a weight-of-evidence approach, and concluded they are expected to be as safe as the four-event stack maize. For some subcombinations that could be produced by conventional crossing through targeted breeding approaches, little or no specific data were submitted, giving rise to uncertainties due to data gaps. To reduce these uncertainties and to confirm assumptions made for the assessment of these subcombinations, the EFSA GMO Panel recommends that the applicant collate relevant information, if these subcombinations were to be created via targeted breeding approaches and commercialised in the future. In this case, this information should focus on expression levels of the newly expressed proteins.

The European Union Reference Laboratory for GM Food and Feed (EURL-GMFF) considers the method validated as fit for the purpose of regulatory compliance. The certified reference materials of maize Bt11, maize MIR162, maize MIR604 and maize GA21 can be accessed at the Institute for Reference Materials and Measurements (IRMM) and at the American Oil Chemists' Society (AOCS).

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. The EFSA GMO Panel is of the opinion that the scope of the PMEM plans provided by the applicant is in line with the scope of the four-event stack maize and the four already assessed stacks. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plans. However, the PMEM plan submitted by the applicant for the four-event stack maize does not include any provision for the six stacks assessed in the scientific opinion (Section 5.2). Therefore, the EFSA GMO Panel recommends the applicant update it accordingly, by following the same aforementioned methodology and reporting policy.

Under the terms of the Regulation (EC) No 1829/2003, the overall opinion fulfils the requirements of Articles 6 and 18 for the placing on the market of genetically modified maize Bt11 × MIR162 × MIR604 × GA21 and subcombinations independently of their origin.

Key words: GMO, overall opinion, maize (*Zea mays*), Bt11, MIR162, MIR604, GA21, herbicide tolerant and insect resistant, stack, Regulation (EC) No 1829/2003

Requestor: On request from the Competent Authority of Germany for an application (EFSA-GMO-DE-2009-66) submitted by Syngenta

Question number: EFSA-Q-2009-00444

Correspondence: gmo@efsa.europa.eu

Acknowledgements: EFSA wishes to thank the GMO Panel members: Andrew Nicholas Birch, Josep Casacuberta, Adinda De Schrijver, Achim Gathmann, 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, Jean-Michel Wal; the members of the Working Groups on Molecular Characterisation, Food and Feed Risk Assessment and Environment Risk Assessment for the preparatory work on this scientific opinion and EFSA staff members: Yi Liu, Zoltán Divéki and Sylvie Mestdagh for the support provided to this scientific output.

Suggested citation: EFSA (European Food Safety Authority), 2015. Overall opinion of the European Food Safety Authority in accordance with Articles 6 and 18 of Regulation (EC) No 1829/2003 on application by Syngenta (reference EFSA-GMO-DE-2009-66) for the placing on the market of 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 supporting publication 2015:EN-909. 10 pp.

Table of contents

1. Introduction

1.1. Background as provided by the Competent Authority of Germany

On 4 March 2009, the European Food Safety Authority (EFSA) received from the Competent Authority of Germany an application (reference EFSA-GMO-DE-2009-66) for authorisation of genetically modified maize Bt11 × MIR162 × MIR604 × GA21 (Unique Identifier SYN-BTØ11-1 × SYN-IR162-4 × SYN-IR6Ø4-5 × MON-ØØØ21-9) and subcombinations independently of their origin submitted by Monsanto within the framework of Regulation (EC) No 1829/2003 on genetically modified food and feed.

The scope defined by the applicant at the time of submission was "*all food and feed products containing, consisting or produced from Bt11 × MIR162 × MIR604 × GA21 maize including products from inbreds and hybrids obtained by conventional breeding of this stacked maize product. The application also covers the import and industrial processing of Bt11 × MIR162 × MIR604 × GA21 maize for all potential uses as any other maize*". On 24 July 2013, the applicant notified that the scope of EFSA-GMO-DE-2009-66 was to "*include Bt11 x MIR 162 x MIR604 x GA21 maize and all subcombinations from Bt11 x MIR 162 x MIR604 x GA21 maize independently of their origin*".

In accordance with Articles 5 and 17 of Regulation (EC) No 1829/2003, EFSA informed the Member States and the European Commission and made the summary of the application publicly available on the EFSA website[3] on 9 March 2009. EFSA initiated a completeness check of the application to check compliance with the requirements laid down in Articles 5 and 17 of Regulation (EC) No 1829/2003. On 19 December 2008, the European Union Reference Laboratory for Genetically modified Food and Feed (EU-RL – GMFF) received samples and control samples in accordance with Articles 5 and 17 of Regulation (EC) No 1829/2003. EFSA declared the application valid on 13 July 2009 and started the clock in accordance with Articles 6 and 18 of Regulation (EC) No 1829/2003.

From that date, EFSA has endeavoured to respect a time limit of six months in giving its overall opinion (Articles 6(1) and 18(1)). EFSA made the valid application available to Member States and the European Commission. Following the procedure laid down in Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, EFSA consulted the Member States. In this context, the Member States risk assessment bodies, as well as the national competent authorities under Directive 2001/18/EC, were given three months after the date of receipt of the valid application (*i.e.* until 22 October 2009) within which to make their opinion known.

Making use of the provisions under Articles 6(2) and 18(2), EFSA requested additional information from the applicant and the clock was stopped from 21 September 2009 to 6 June 2012, from 6 July 2012 to 27 September 2013 and from 5 February 2014 to 23 October 2015[4].

The overall opinion on application EFSA-GMO-DE-2009-66 includes the scientific opinion of the Scientific Panel on Genetically Modified Organisms together with the particulars required under Articles 6(5)(a-g) and 18(5)(a-g) of Regulation (EC) No 1829/2003: i) the name and address of the applicant, ii) the designation of the food and its specification, iii) the information required under Annex II to the Cartagena Protocol, iv) the labelling proposal, v) the method for detection, validated by the European Union Reference Laboratory, including sampling, identification of the transformation event in the food-

[3] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2009-00444

[4] Requests for supplementary information from the EFSA GMO Panel: Requested(1) on 21/09/2009 – received on 21/12/2009; requested(2) on 05/02/2010 – received on 05/10/2010; requested(3) on 17/03/2010 – received on 03/06/2010; requested(4) on 21/01/2011 – received on 01/02/2012 and clock re-started on 06/06/2012. Requested(5) on 06/07/2012 – received on 10/10/2012; requested(6) on 07/12/2012 – received on 19/03/2013 and on 25/03/2013; requested(7) on 05/02/2013 – received on 25/03/2013, requested(8) on 20/03/2013 – received on 25/03/2013 and on and clock re-started on 27/09/2013. Requested(9) on 05/02/2014 – received on 18/02/2014; requested(10) on 13/03/2014 – received on 16/06/2014 and on 28/07/2014; requested(11) on 09/09/2014 – received on 25/09/2014; requested(12) on 16/09/2014 – received on 15/10/2014; requested(13) on 24/10/2014 – received on 03/07/2015; requested(14) on 18/09/2015 – received on 24/09/2015 and clock re-started on 23/10/2015.

Request for clarifications from the applicant: Requested(1) on 27/05/2015 – clarifications provided by EFSA on 05/06/2015.

Additional information was submitted spontaneously by the applicant: on 10/12/2013, 28/07/2014, 21/07/2015 and 10/08/2015.

feed and/or foods-feeds produced from it, vi) an indication of where appropriate reference materials can be accessed, vii) the monitoring plan and viii) the Member States' comments submitted during the three-month consultation period.

1.2. Terms of Reference

The European Food Safety Authority (EFSA) received from the Competent Authority of Germany an application for authorisation of genetically modified maize Bt11 × MIR162 × MIR604 × GA21 (Unique Identifier SYN-BTØ11-1 × SYN-IR162-4 × SYN-IR6Ø4-5 × MON-ØØØ21-9) submitted by Syngenta within the framework of Regulation (EC) No 1829/2003 on genetically modified food and feed (reference EFSA-GMO-DE-2009-66). On 24 July 2013), the applicant notified that the scope of EFSA-GMO-DE-2009-66 was to "*include Bt11 x MIR 162 x MIR604 x GA21 maize and all subcombinations from Bt11 x MIR 162 x MIR604 x GA21 maize independently of their origin*". EFSA was requested to issue an overall opinion in line with the requirements of the Regulation (EC) No 1829/2003 (Articles 6 and 18).

2. Considerations

2.1. Applicant

The application was submitted by

Syngenta Crop Protection AG
Schwarzwaldallee 215
CH 4058 Basel
Switzwerland

2.2. Designation and specification of the product

The scope defined by the applicant at the time of submission was "*all food and feed products containing, consisting or produced from Bt11 × MIR162 × MIR604 × GA21 maize including products from inbreds and hybrids obtained by conventional breeding of this stacked maize product. The application also covers the import and industrial processing of Bt11 × MIR162 × MIR604 × GA21 maize for all potential uses as any other maize.*" After clarifications (letters dated 14 June 2010, 15 September 2010, 15 March 2012, and 6 June 2012, 8 July 2013 and 24 July 2013), the applicant notified EFSA that the scope of EFSA-GMO-DE-2009-66 was to "*include Bt11 x MIR 162 x MIR604 x GA21 maize and all subcombinations from Bt11 x MIR 162 x MIR604 x GA21 maize independently of their origin*".

The four-event stack maize Bt11 × MIR162 × MIR604 × GA21 was produced by conventional crossing to combine four single maize events. Maize containing the single events, Bt11 (expressing Cry1Ab and PAT proteins), MIR162 (expressing Vip3Aa20 and PMI proteins), MIR604 (expressing mCry3A and PMI proteins) and GA21 (expressing mEPSPS protein). The four-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 pests (e.g. *Ostrinia nubilalis* (European corn borer) and *Sesamia nonagrioides* (Mediterranean corn borer)) and coleopteran pests (*Diabrotica* spp. (corn rootworm)).

3. Scientific opinion of the EFSA GMO Panel

In delivering its scientific opinion, the EFSA GMO Panel considered the data available on the four-event stack maize and the subcombinations, the scientific comments submitted by the Member States and the relevant scientific publications. The Scientific Panel on Genetically Modified Organisms (EFSA GMO Panel) has carried out the scientific assessment of GM maize Bt11 × MIR162 × MIR604 × GA21 and subcombinations independently of their origin in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. The EFSA GMO Panel previously assessed the four single events combined to produce a four-event stack maize Bt11 × MIR162 × MIR604 × GA21 and did not identify safety concerns. In this opinion, the EFSA GMO Panel assesses the four-event stack maize and all its subcombinations independently of their origin. No new data on the single events, leading to modification of the original conclusions on their safety, were identified. The molecular, agronomic, phenotypic and compositional data on the four-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 four-event stack maize. Considering the routes of exposure and limited exposure levels, the Panel concludes that this four-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 four-event stack maize is as safe and as nutritious as its conventional counterpart in the context of its scope. Among the 10 subcombinations, four have been assessed previously and no safety concerns were identified. For the remaining six subcombinations, the EFSA GMO Panel followed a weight-of-evidence approach, and concluded they are expected to be as safe as the four-event stack maize. For some subcombinations that could be produced by conventional crossing through targeted breeding approaches, little or no specific data were submitted, giving rise to uncertainties due to data gaps. To reduce these uncertainties and to confirm assumptions made for the assessment of these subcombinations, the EFSA GMO Panel recommends that the applicant collate relevant information, if these subcombinations were to be created via targeted breeding approaches and commercialised in

the future. In this case, this information should focus on expression levels of the newly expressed proteins (Annex A).

4. Cartagena Protocol

The EFSA GMO Panel was not requested to give an opinion on information required under Annex II to the Cartagena Protocol, which is a matter related to risk management (Annex B).

5. Labelling

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. (Annex C).

6. Method for detection

The Joint Research Centre (JRC) as Community Reference Laboratory for the Genetically Modified Food and Feed has carried out a collaborative study to assess the performance of four quantitative event-specific methods on the hybrid maize line Bt11 × MIR162 × MIR604 × GA21 which combines the Bt11, the MIR162, the MIR604 and the GA21 transformation events. The four methods have been validated individually on single-trait events, to detect and quantify each event in maize samples. The reports were issued on 3 April 2007, 20 June 2008, 30 March 2010, 31 January 2011 and 11 December 2013. These methods, developed and validated to detect and quantify the single events, can be equally applied for the quantification of the respective events combined in GM maize stack Bt11 × MIR162 × MIR604 × GA21 (Annexes D1, D2a, D2b, D2c and D2d).

7. Certified reference materials

The certified reference materials of maize Bt11, maize MIR162, maize MIR604 and maize GA21 can be accessed at the Institute for Reference Materials and Measurements (IRMM) and at the American Oil Chemists' Society (AOCS) (Annexes E1, E2, E3 and E4).

8. Post-market environmental monitoring (PMEM)

The EFSA GMO Panel is of the opinion that the scope of the PMEM plans provided by the applicant is in line with the scope of the four-event stack maize and the four already assessed stacks. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plans. However, the PMEM plan submitted by the applicant for the four-event stack maize does not include any provision for the six stacks assessed in the scientific opinion (Section 5.2). Therefore, the EFSA GMO Panel recommends the applicant update it accordingly, by following the same aforementioned methodology and reporting policy (Annex F).

9. Member States' Comments[5]

Issues raised by Member States on maize Bt11 × MIR162 × MIR604 × GA21 were considered in the scientific opinion and are addressed in detail in Annex G of the EFSA overall opinion (Annex G).

[5] The Member States' Comments (Annex G) contains comments for the four-event stack maize Bt11 x MIR162 x MIR604 x GA21, but also for the single event MIR162. This is because that the initial submission (4 March 2009) of application EFSA-GMO-DE-2009-66 covered three events (Bt11 x MIR162 x MIR604 x GA21, Bt11 x MIR604 x GA21 and MIR162), and this MS comments were collected during the period from 21 July 2009 to 22 October 2009. Only later (23 July 2010), the single event MIR162 was submitted separately as application EFSA-GMO-DE-2010-82, and was no longer part of the application EFSA-GMO-DE-2009-66. EFSA assessed MIR162 (EFSA Journal 2012;10(6):2756 [27 pp.]), and addressed MIR162-specific MS comments in the Annex G of the EFSA overall opinion for maize MIR162.

10. Conclusions

Under the terms of the Regulation (EC) No 1829/2003, the overall opinion fulfils the requirements of Articles 6 and 18 for the placing on the market of genetically modified maize Bt11 × MIR162 × MIR604 × GA21 and subcombinations independently of their origin.

List of Annexes[6]

Annex A:	Scientific opinion of the EFSA GMO Panel (maize Bt11 × MIR162 × MIR604 × GA21 and subcombinations independently of their origin)
Annex B:	Cartagena Protocol (maize Bt11 × MIR162 × MIR604 × GA21 and subcombinations independently of their origin)
Annex C:	Labelling (maize Bt11 × MIR162 × MIR604 × GA21 and subcombinations independently of their origin)
Annex D1:	Validation report (maize Bt11 × MIR162×MIR604 × GA21)
Annex D2a:	Validated method (maize Bt11)
Annex D2b:	Validated method (maize MIR162)
Annex D2c:	Validated method (maize MIR604)
Annex D2d:	Validated method (maize GA21)
Annex D3:	DNA Extraction (maize Bt11 × MIR162 × MIR604 × GA21)
Annex E1:	Certified reference materials (maize Bt11)
Annex E2:	Certified reference materials (maize MIR162)
Annex E3:	Certified reference materials (maize MIR604)
Annex E4:	Certified reference materials (maize GA21)
Annex F:	Post-market environmental monitoring (maize Bt11 × MIR162 × MIR604 × GA21 and maize Bt11 x MIR162 x GA21)
Annex G:	Member States' comments (maize Bt11 × MIR162 × MIR604 × GA21)

[6] The annexes of the EFSA overall opinion can be found in the Register of Questions (tab "Question documents") on the EFSA website under the following link: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2015-00651