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SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 31 May 2018

doi: 10.2903/j.efsa.2018.5310

Assessment of genetically modified maize MON 87411 for food and feed uses, import and processing, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2015-124)

EFSA Panel on Genetically Modified Organisms (GMO),
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Abstract

Maize MON 87411 was developed to confer resistance to corn rootworms (*Diabrotica* spp.) by the expression of a modified version of the *Bacillus thuringiensis cry3Bb1* gene and a DvSnf7 dsRNA expression cassette, and tolerance to glyphosate-containing herbicides by the expression of a CP4 5-enolpyruvylshikimate-3-phosphate synthase (*cp4 epsps*) gene. The molecular characterisation data and bioinformatics analyses did not identify issues requiring assessment for food and feed safety. No statistically significant differences in the agronomic and phenotypic characteristics tested between maize MON 87411 and its conventional counterpart were identified. The compositional analysis of maize MON 87411 did not identify differences that required further assessment except for palmitic acid levels in grains from not treated maize MON 87411. The GMO Panel did not identify safety concerns regarding the toxicity and allergenicity of the Cry3Bb1 and CP4 EPSPS proteins, as expressed in maize MON 87411 and found no evidence that the genetic modification might significantly change the overall allergenicity of maize MON 87411. The nutritional impact of maize MON 87411-derived food and feed is expected to be the same as those derived from the conventional counterpart and non-GM commercial reference varieties. The GMO Panel concludes that maize MON 87411, as described in this application, is nutritionally equivalent to and as safe as the conventional counterpart and the non-GM maize reference varieties tested, and no post-market monitoring of food/feed is considered necessary. In the case of accidental release of viable maize MON 87411 grains into the environment, maize MON 87411 would not raise environmental safety concerns. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of maize MON 87411. The GMO Panel concludes that maize MON 87411, as described in this application, is as safe as its conventional counterpart and the tested non-GM maize reference varieties with respect to potential effects on human and animal health and the environment.

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Keywords: GMO, maize (*Zea mays*), MON 87411, Regulation (EC) No 1829/2003, DvSnf7, Cry3Bb1, CP4 EPSPS

Requestor: Competent Authority of the Netherlands

Question number: EFSA-Q-2015-00101

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Acknowledgements: The Panel wishes to thank the members of the Working Group on Molecular Characterisation, Food and Feed Safety Assessment and Environmental Risk Assessment and Yann Devos, Irene Muñoz Guajardo and Claudia Paoletti for the preparatory work on this scientific output.

Suggested citation: EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Birch AN, Casacuberta J, De Schrijver A, Gralak AM, Guerche P, Jones H, Manachini B, Messéan A, Nielsen EE, Nogué F, Robaglia C, Rostoks N, Sweet J, Tebbe C, Visioli F, Wal J-M, Ardizzone M, De Sanctis G, Fernandez Dumont A, Gennaro A, Gómez Ruiz JA, Lanzoni A, Neri FM, Papadopoulou N, Paraskevopoulos K and Ramon M, 2018. Scientific Opinion on the assessment of genetically modified maize MON 87411 for food and feed uses, import and processing, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2015-124). EFSA Journal 2018;16(6):5310, 29 pp. https://doi.org/10.2903/j.efsa.2018.5310

ISSN: 1831-4732

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Summary

Following the submission of application EFSA-GMO-NL-2015-124 under Regulation (EC) No 1829/2003[1] from Monsanto, the Panel on Genetically Modified Organisms of the European Food Safety Authority (GMO Panel) was asked to deliver a scientific opinion on the safety of the genetically modified (GM) maize (*Zea mays* L.) MON 87411 (unique identifier MON-87411-9). The scope of application EFSA-GMO-NL-2015-124 is for import, processing, and food and feed uses of maize MON 87411 within the European Union (EU), but excludes cultivation in the EU.

The GMO Panel evaluated maize MON 87411 with reference to the scope and appropriate principles described in Regulation (EU) 503/2013[2] and its guidelines for the risk assessment of GM plants. The evaluation addressed the following components of the risk assessment: the molecular characterisation of the inserted DNA, analysis of the expression of the corresponding proteins and an in planta RNAi off-target screen; the comparative analyses of compositional, agronomic and phenotypic characteristics; the safety of the newly expressed proteins, the DvSnf7 dsRNA expression cassette and the whole food and feed with respect to potential toxicity, allergenicity, nutritional characteristics and dietary exposure; the environmental risk assessment; and the post-market environmental monitoring (PMEM) plan.

The molecular characterisation data establish that maize MON 87411 contains a single insert consisting of one copy of the *cp4 epsps* and the *cry3Bb1* expression cassettes and one copy of the DvSnf7 dsRNA expression cassette. Bioinformatics analyses of the sequences encoding the newly expressed proteins and other open reading frames within the insert, or spanning the junctions between the insert and genomic DNA, do not indicate significant similarities to toxins and allergens. The in planta RNAi off-target search, performed with the sequence of the DvSnf7 dsRNA, does not provide indication for an off-target effect that would need further safety assessment. The stability of the inserted DNA and of the introduced trait is confirmed over several generations. The methodology used to quantify the levels of the CP4 EPSPS and Cry3Bb1 proteins is considered adequate. The protein characterisation data comparing the structural and biochemical properties of plant and microbial derived CP4 EPSPS and Cry3Bb1 proteins indicate that these proteins are equivalent and the microbial produced proteins can be used in the safety studies.

No statistically significant differences in the agronomic, phenotypic and physiological characteristics between maize MON 87411 and its conventional counterpart are identified. None of the differences identified in forage and grain composition between maize MON 87411, its conventional counterpart and the non-GM commercial reference varieties needs further assessment regarding food and feed safety, except for palmitic acid levels in grains from not treated maize MON 87411, which were further assessed.

The GMO Panel did not identify safety concerns regarding the toxicity and allergenicity of the CP4 EPSPS and Cry3Bb1 proteins, as expressed in maize MON 87411 and found no evidence that the genetic modification might significantly change the overall allergenicity of maize MON 87411. The nutritional impact of maize MON 87411-derived food and feed is expected to be the same as those derived from the conventional counterpart and non-GM commercial reference varieties. The GMO Panel concludes that maize MON 87411, as described in this application, is nutritionally equivalent to and as safe as the conventional counterpart and the non-GM maize reference varieties tested, and no post-market monitoring of food/feed is considered necessary.

Considering the introduced traits, the outcome of the comparative analysis, the routes and levels of exposure, the GMO Panel concludes that maize MON 87411 would not raise safety concerns in the case of accidental release of viable GM maize grains into the environment. The PMEM plan and reporting intervals are in line with the intended uses of maize MON 87411.

Based on the relevant publication identified through the literature searches, the GMO Panel did not identify any safety issues pertaining to the intended uses of maize MON 87411. In the context of PMEM, the applicant should improve future literature searches according to the GMO Panel recommendations.

In delivering its scientific opinion, the GMO Panel took into account application EFSA-GMO-NL-2015-124, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications. The GMO Panel concludes that maize MON 87411, as described in this application, is as safe as its conventional counterpart and the tested non-GM maize reference varieties with respect to potential effects on human and animal health and the environment.

[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] Commission 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.

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1. Introduction

The scope of application EFSA-GMO-NL-2015-124 is for food and feed uses, import and processing of maize MON 87411 and does not include cultivation in the European Union (EU).

Maize MON 87411 was developed to confer resistance to corn rootworms (CRW) (*Diabrotica* spp.) and tolerance to glyphosate-containing herbicides. Resistance to CRW is achieved by the expression of a modified version of the *Bacillus thuringiensis* (subsp. *kumamotoensis*) Cry3Bb1 protein and the expression of a DvSnf7 dsRNA, down-regulating the *Snf7* gene transcript in the insect via RNA interference (RNAi), leading to pest mortality after consumption. Tolerance to glyphosate was achieved by the expression of CP4 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS), which has a reduced affinity for glyphosate and maintains enzymatic activity in its presence.[3]

1.1. Background

On 10 February 2015, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands application EFSA-GMO-NL-2015-124 for authorisation of maize MON 87411 (Unique Identifier MON-87411-9), submitted by Monsanto Europe within the framework of Regulation (EC) No 1829/2003 on GM food and feed.

After receiving application EFSA-GMO-NL-2015-124, and in accordance with Articles 5(2)(b) and 17 (2)(b) of the 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.[4] EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of the Regulation (EC) No 1829/2003 and Regulation (EU) No 503/2013. On 23 April 2015 and on 27 July 2015, EFSA received additional information requested under completeness check on 25 March 2015. On 17 August 2015, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the European Commission, and consulted nominated risk assessment bodies of Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC[5] following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Member States had three months after the date of receipt of the valid application (until 26 November 2015) to make their opinion known.

The GMO Panel requested additional information from the applicant on 8 September 2015, 10 November 2015, 21 December 2015 and 11 February 2016. The applicant provided information on 20 November 2015, on 22 March 2016 and on 3 May 2016 respectively; the clock re-started on 3 May 2016. The GMO Panel requested additional information from the applicant on 13 May 2016[6]; the applicant provided information on 27 June 2016; the clock re-started on 27 June 2016. The GMO Panel requested additional information from the applicant on 1 July 2016; the applicant provided information on 11 July 2016; the clock re-started on 11 July 2016. The GMO Panel requested additional information from the applicant on 20 July 2016[7]; the applicant provided information on 22 September 2016; the clock re-started on 22 September 2016. The GMO Panel requested additional information from the applicant on 26 September 2016; the applicant provided information on 24 November 2016; the clock re-started on 24 November 2016. The GMO Panel requested additional information from the applicant on 5 December 2016; the applicant provided information on 10 January 2017; the clock re-started on 10 January 2017. The GMO Panel requested additional information from the applicant on 19 January 2017[8]; the GMO Panel requested additional information from the applicant on 29 March 2017; the GMO Panel requested additional information from the applicant on 3 May 2017; the applicant provided information on 30 May 2017, 15 June 2017 and on 19 June 2017; the clock re-started on 19 June 2017. The GMO Panel requested additional information from the applicant on 6 July 2017; the GMO Panel requested additional information from the applicant on 19 July 2017; the GMO Panel requested additional information from the applicant on 2 August 2017; the applicant provided information on 22 August 2017; the GMO Panel requested

[3] Dossier: Part II – Section 1.2.2.1.
[4] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2015-00101
[5] 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.
[6] The applicant requested clarifications on 13 May 2016; EFSA provided the clarifications requested on 23 May 2016.
[7] The applicant provided additional information spontaneously on 16 September 2016.
[8] EFSA requested clarifications on 22 February 2017; the applicant provided the clarifications requested on 7 March 2017.

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additional information from the applicant on 22 September 2017; the applicant provided information on 6 October 2017; the GMO Panel requested additional information from the applicant on 7 November 2017; the GMO Panel requested additional information from the applicant on 4 December 2017; the applicant provided information on 20 December 2017; the GMO Panel requested additional information from the applicant on 17 January 2018; the applicant provided information on 5 February 2018 and on 28 February 2018; the clock re-started on 28 February 2018. The GMO Panel requested additional information from the applicant on 28 March 2018[9]; the applicant provided information on 6 April 2018; the clock re-started on 6 April 2018; the applicant provided additional information spontaneously on 31 May 2018.

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

In giving its scientific opinion on maize MON 87411 to the European Commission, Member States and the applicant, and in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003, EFSA has endeavoured to respect a time limit of six months from the acknowledgement of the valid application. As additional information was requested by the GMO Panel, the time limit of six 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, this Scientific Opinion is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation and thus will be part of the EFSA overall opinion in accordance with Articles 6(5) and 18(5).

1.2. Terms of Reference as provided by the requestor

The GMO Panel was requested to carry out a scientific assessment of maize MON 87411 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003.

Where applicable, any conditions or restrictions for 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 and feed containing or consisting of GMOs, conditions for the protection of particular ecosystems/environment and/or geographical areas should be indicated in accordance with Articles 6(5)(e) and 18(5)(e) of Regulation (EC) No 1829/2003.

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

2. Data and methodologies

2.1. Data

In delivering its Scientific Opinion, the GMO Panel took into account application EFSA-GMO-NL-2015-124, 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 MON 87411 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 Regulation (EU) No 503/2013 and the appropriate principles described in its guidelines for the risk assessment of GM plants and derived food and feed (EFSA GMO Panel, 2011a), the environmental risk assessment (ERA) of GM plants (EFSA GMO Panel, 2010a), and the post-market environmental monitoring (PMEM) of GM plants (EFSA GMO Panel, 2011b).

The GMO Panel took into account the criteria included in the EFSA Scientific Committee (2011) guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed for GMO risk assessment' and in the 'Explanatory statement for its applicability (EFSA, 2014), to perform the assessment of the 90-day feeding study provided.

[9] The applicant provided additional information spontaneously on 5 April 2018.

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

The comments raised by Member States are addressed in Annex G of EFSA's overall opinion[10] and were taken into consideration during the scientific risk assessment.

3. Assessment

3.1. Systematic literature review[11]

The GMO Panel assessed the applicant's literature searches on maize MON 87411, which include a scoping review, according to the guidelines given in EFSA (2010, 2017).

A systematic review as referred to in the Regulation (EU) No 503/2013 has not been provided in support to the risk assessment of the application EFSA-GMO-NL-2015-124. Based on the outcome of the scoping review, the GMO Panel agrees that there is limited value of undertaking a systematic review for maize MON 87411 at present.

Although the overall quality of the performed literature searches is acceptable, the GMO Panel considers that future searches on maize MON 87411 could be improved. The GMO Panel therefore recommends the applicant to:

- design broader and more sensitive searches in the context of maize MON 87411, in order to identify as many relevant publications as possible;
- ensure that enough search term variation is used (covering possible synonyms, related terms, acronyms, spelling variants, old and new terminology, brand and generic names, lay and scientific terminology, common typos, translation issues);
- use truncation consistently;
- adapt the search to the size of the identified publications (and thus not combine search sets when one of the search sets already yields only a small number of publications);
- assess the relevance and risk assessment implications of publications retrieved via searches beyond electronic bibliographic databases.

The literature searches did not identify relevant publications that were not already submitted as part of the application.

3.2. Molecular characterisation

3.2.1. Transformation process and vector constructs

Maize MON 87411 was developed by *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*)-mediated transformation. Immature embryos of maize line LH244 were co-cultured with a disarmed *A. tumefaciens* strain ABI containing the vector PV-ZMIR10871.[12] The plasmid PV-ZMIR10871 used for the transformation contains three expression cassettes between the right and left border of the T-DNA,[13] containing the following genetic elements:

- The DvSnf7 dsRNA expression cassette consists of the *e35S* promoter from *Cauliflower Mosaic Virus*, the heat shock protein 70 intron from *Zea mays*, two fragments of the coding sequence of the *Snf7* gene in an inverted repeat configuration and the 3′ untranslated sequence of the *E9* gene from *Pisum sativum.*
- The *cry3Bb1* expression cassette contains the *pIIG* promoter from *Zea mays*, the chloroplyll a/b binding protein leader from *Triticum aestivum*, the *RactI* intron from *Oryza sativa*, the codon optimised *cry3Bb1* coding sequence from *Bacillus thuringiensis*, and the heat shock protein 17 3′ untranslated region from *T. aestivum*.
- The *cp4 epsps* expression cassette consists of the *TubA* promoter, leader and intron from *O. sativa*, the *CTP2* target sequence of the *shkG* gene from *Arabidopsis thaliana*, the codon-optimised CP4 *epsps* coding sequence of the *aroA* gene from *Agrobacterium* sp. strain CP4, and the 3′ untranslated region of the *TubA* gene from *O. sativa*.

[10] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2018-00468
[11] Dossier: Part II – Section 7.
[12] Dossier: Part II - Section 1.2.1.1.
[13] Dossier: Part II - Section 1.2.1.2.

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The vector backbone contained elements necessary for the maintenance and selection of the plasmid in bacteria.

3.2.2. Transgene constructs in the GM plant

Molecular characterisation of maize MON 87411 was performed by next generation sequencing (NGS) and junction sequence analysis (JSA), polymerase chain reaction (PCR) and DNA sequence analysis in order to determine insert copy number, size and organisation of the inserted sequences and to confirm the absence of plasmid backbone sequences.[14] The approach used was acceptable in terms of coverage and sensitivity.

NGS/JSA of the whole genome indicated that maize event MON 87411 contains a single insert, consisting of a single copy of the T-DNA in the same configuration as in the PV-ZMIR10871 transformation vector. NGS/JSA also indicated the absence of vector backbone sequences.

The nucleotide sequence of the entire insert of maize MON 87411 together with 1,460 bp of the 5′ and 1,802 bp of the 3′ flanking regions were determined. The insert of 11,248 bp is identical to the T-DNA of PV-ZMIR10871, except for the deletion of 316 bp of the right border region and 179 bp of the left border region.[14]

A comparison with the pre-insertion locus indicated that 118 bp were deleted from the maize genomic DNA.[14] The possible interruption of known endogenous maize genes by the insertion in maize MON 87411 was evaluated by bioinformatics analyses of the pre-insertion locus and of the genomic sequences flanking the insert.[14] The results of these analyses did not indicate the interruption of any known endogenous gene in maize MON 87411.

The results of segregation (see Section 3.2.5) and bioinformatics analyses established that the insert is located in the nuclear genome.

Updated bioinformatics analyses of the amino acid sequence of the newly expressed CP4 EPSPS and Cry3Bb1 proteins revealed no significant similarities to toxins and allergens. In addition, updated bioinformatics analyses of the newly created open reading frames (ORFs) within the insert and spanning the junctions between the insert and genomic DNA did not indicate significant similarities to toxins and allergens.[15]

According to Regulation (EU) No 503/2013, when silencing approaches by RNAi have been used in GM plant applications, a bioinformatics analysis to identify potential 'off target' genes is required. The applicants have followed the recommendations by the EFSA GMO Panel for an RNAi off-target search in the plant expressing the dsRNA.[16,17] None of the maize transcript sequences present in the available databases showed perfect match to any of the siRNAs potentially produced. Few maize transcript sequences had regions matching to those siRNAs with one to four mismatches. Some of these sequences presented matches for more than one potential siRNA (up to five). The applicant discussed these results, taking into account the potential function of the proteins encoded by the mRNAs matching the siRNAs. The GMO Panel assessed this information and concludes that it does not provide indication for an off-target effect of the DvSnf7 dsRNA expression that would need further safety assessment.

In order to assess the possibility for horizontal gene transfer (HGT) by homologous recombination (HR), the applicant performed a sequence identity analysis of the regions of bacterial origin in maize MON 87411. The likelihood and potential consequences of plant-to-bacteria gene transfer are described in Section 3.5.1.2.

3.2.3. Protein characterisation and equivalence

Maize MON 87411 expresses two new proteins, Cry3Bb1 and CP4 EPSPS. Given the technical restraints in producing large enough quantities from plants, these proteins were recombinantly produced in *Escherichia coli*. A set of biochemical methods was employed to demonstrate the equivalence between the maize and E. *coli*-derived Cry3Bb1 and CP4 EPSPS. Purified proteins from these two sources were characterised and compared in terms of their physicochemical, structural and functional properties.

[14] Dossier: Part II - Section 1.2.2.2.
[15] Dossier: Part II - Section 1.2.2.2; Additional information: 22/3/2016 and 6/10/2017.
[16] Annex II of the minutes of the 118th GMO plenary meeting (https://www.efsa.europa.eu/sites/default/files/event/171025-m.pdf)
[17] Dossier: Part II – Section 1.2.1.3; Report RAR-2015-0373; Additional information: 22/3/2016, 19/6/2017, 20/12/2017 and 28/2/2018; spontaneous information 5/4/2018.

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Cry3Bb1 protein characterisation and equivalence[18]

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis showed that both plant and microbe-derived Cry3Bb1 proteins had the expected molecular weight of ~ 74 kDa and were comparably immunoreactive to Cry3Bb1 protein-specific antibodies. An additional ~ 65 kDa truncated form of the Cry3Bb1 protein in the plant-derived sample was also identified by western blot analysis. Glycosylation detection analysis demonstrated that none of the Cry3Bb1 proteins were glycosylated. Amino acid sequence analysis of the intact plant-derived Cry3Bb1 protein by mass spectrometry (MS) methods showed that the protein matched the deduced sequence as defined by the *cry3Bb1* gene. These sequence analysis data were consistent with the previously analysed microbe-derived intact Cry3Bb1 protein. In addition, the MS data showed that the N-terminal methionine was truncated and alanine too was acetylated. Such modifications are common in eukaryotic proteins (e.g. Polevoda and Sherman, 2000). N-terminal sequence analysis of the plant-derived Cry3Bb1 truncated form showed that the first 49 amino acids were truncated. Functional equivalence was demonstrated by an insect feeding bioassay which showed that plant and microbe-derived Cry3Bb1 protein samples had comparable insecticidal activity.

CP4 EPSPS protein characterisation and equivalence[19]

SDS-PAGE analysis and Western blot analysis showed that both plant and microbe-derived CP4 EPSPS proteins had the expected molecular weight of ~ 47.5 kDa and were comparably immunoreactive to CP4 EPSPS protein-specific antibodies. Glycosylation detection analysis demonstrated that none of the CP4 EPSPS proteins were glycosylated. Amino acid sequence analysis by MS methods showed that the plant-derived protein matched the deduced sequence as defined by the inserted *cp4 epsps* gene. In addition, N-terminal sequence analysis showed that the N-terminal methionine of the plant-derived CP4 EPSPS was truncated. Such modifications are common in eukaryotic proteins (e.g. Polevoda and Sherman, 2000). These sequence analysis data were consistent with the previously analysed microbe-derived CP4 EPSPS protein. Functional equivalence was demonstrated by a biochemical *in vitro* activity assay that showed that both proteins had comparable activity.

The protein characterisation data comparing the structural, biochemical and functional properties of plant and microbe-derived Cry3Bb1 and CP4 EPSPS proteins indicate that these proteins are equivalent. Therefore, the GMO Panel accepts the use of the Cry3Bb1 and CP4 EPSPS proteins produced in bacteria for the safety studies.

3.2.4. Information on the expression of the insert

Protein levels of CP4 EPSPS and Cry3Bb1 were analysed by an enzyme-linked immunosorbent assay (ELISA) in material harvested in a field trial across five locations in Argentina during the 2011–2012 growing season. Samples analysed included OSL1-4, OSR1-4, OSWP1-4, stover, senescent root, forage root, forage, grain, pollen and silk tissue from plants treated with the intended herbicide and forage and grain from plants not treated with the intended herbicide.[20] The mean values, standard deviations and ranges of protein expression levels in grains (n = 20) and forage (n = 20) of the CP4 EPSPS and Cry3Bb1 proteins are summarised in Table 1.

The applicant provided a measure of the levels of DvSnf7 dsRNA in different tissues including grain and forage. However, the dsRNA is an intermediate molecule which is processed by dicer to siRNA molecules and the levels of dsRNA are not a good proxy for the levels of the active siRNAs in the plant (Pačes et al., 2017). Therefore, the levels of the DvSnf7 dsRNA were not considered relevant for the risk assessment of maize MON 87411.

[18] Dossier: Part II – Section 1.4.1.1(a) and Study: MSL0024872.
[19] Dossier: Part II – Section 1.4.1.1(b) and Study: MSL0024834.
[20] Dossier: Part II - Section 1.2.2.3.

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Table 1: Means, standard deviations and ranges of protein levels in grains and forage (μg/g dry weight) from maize MON 87411

Tissue	Glyphosate treatment	
	Untreated	Treated
Grain		
CP4 EPSPS	2.3[(a)] ± 0.47[(b)] (1.5 – 3.2)[(c)]	1.9 ± 0.31 (1.6 – 3.1)
Cry3Bb1	3.3 ± 0.47 (2.2 – 4.2)	4.0 ± 0.56 (3.1 – 5.1)
Forage		
CP4 EPSPS	7.1 ± 2.6 (4.1 – 13)	8.0 ± 2.3 (5.2 – 13)
Cry3Bb1	35 ± 16 (15 – 69)	39 ± 17 (18 – 75)

(a): Mean value.
(b): Standard deviation.
(C): Range.

3.2.5. Inheritance and stability of inserted DNA

Genetic stability of maize MON 87411 insert was assessed by NGS/JSA for five generations and PCR-based segregation analysis from three generations. The results indicated that all the plants tested retained the single copy of the insert and flanking regions, which were stably inherited in subsequent generations. The results supported the presence of a single insertion, segregating in a Mendelian fashion.[21]

3.2.6. Conclusion on molecular characterisation

The molecular characterisation data establish that maize MON 87411 contains a single insert consisting of one copy of the *cp4 epsps* and the *cry3Bb1* expression cassettes and one copy of the DvSnf7 dsRNA expression cassette. Bioinformatics analyses of the sequences encoding the newly expressed proteins and other ORFs within the insert or spanning the junctions between the insert and genomic DNA do not indicate significant similarities to toxins and allergens. The in planta RNAi off-target search, performed with the sequence of the DvSnf7 dsRNA, do not provide indication for an off-target effect that would require further safety assessment. The stability of the inserted DNA and of the introduced trait is confirmed over several generations. The methodology used to quantify the levels of the CP4 EPSPS and Cry3Bb1 proteins is considered adequate. The protein characterisation data comparing the structural and biochemical properties of plant- and microbe-derived CP4 EPSPS and Cry3Bb1 proteins indicate that these proteins are equivalent and the microbe-produced protein can be used in safety studies.

3.3. Comparative analysis[22]

3.3.1. Choice of comparator and production of material for the comparative assessment[23]

Application EFSA-GMO-NL-2015-124 presents data on agronomic and phenotypic characteristics, as well as on forage and grain composition of maize MON 87411 derived from a field trial study performed at nine sites in Argentina during the 2011–2012 growing season (Table 2).[24] In addition, the application contains data on characteristics of maize MON 87411 pollen and seed.

[21] Dossier: Part II - Section 1.2.2.4; Additional information 22/8/2017 and 20/12/2017.
[22] Dossier: Part II – Section 1.3; Additional information 27/6/2016, 11/7/2016.
[23] Dossier: Part II – Section 1.3.1.
[24] For agronomic and phenotypic characteristics, one additional site from Argentina and from the same growing season was submitted; additional information: 27/6/2016, 11/7/2016.

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Table 2: Overview of comparative assessment studies with maize MON 87411 provided in application EFSA-GMO-NL-2015-124

Study focus	Study details	Conventional counterpart	Commercial non-GM reference varieties
Agronomic, phenotypic analysis	Field trials 2011/12, Argentina (9 locations)	LH244xHCL645	23
	Seed characteristics conducted in 2012 under controlled conditions	LH244xLH287(a)	11
	Pollen morphology and viability study 2012, 1 site located in the US	LH244xLH287(b)	4
Compositional analysis	Field trials 2011/12, Argentina (8 locations)	LH244xHCL645	22

(a): Grains collected from three sites located in the US.
(b): Pollen collected from one site located in the US.

The field trial study for the agronomic, phenotypic and compositional assessment of maize MON 87411 was conducted during the 2011–2012 growing season in the major maize-growing areas in Argentina.[25] The sites covered a small geographical range, and consequently were similar in soil characteristics and climate conditions. The representativeness of these sites was questioned (EFSA GMO Panel, 2015a) as it was doubtful whether they would capture the variability that may exist across potential receiving environments for the commercial cultivation of this GM maize line. Upon request of the EFSA GMO Panel, a rationale for the site selection[26] and one additional site from Argentina,[27] from the same growing season, was submitted for agronomic and phenotypic characteristics. The updated dataset was considered to conform to the EFSA guidance (2011a). At each site, the following materials were included: plots containing GM plants exposed to the intended herbicide (glyphosate) in addition to the conventional herbicides, plots with comparator plants treated with conventional herbicide management regimes and plots with GM plants treated with the same conventional herbicide management regimes. In total, across all the sites, 23 non-GM maize reference varieties[28] were included in the field trial study.

Maize event MON 87411 was obtained using the non-GM maize line LH244 as recipient inbred line (see Section 3.2.1). As documented by the pedigree, two different breeding strategies were followed to produce the hybrid used in the field trial study (Argentina) and the hybrid used to analyse the seed germination and the pollen characteristics (US). For the field trial study the transformed inbred line LH244 was crossed with the non-GM inbred line HCL645 to produce the GM hybrid used to conduct the agronomic and phenotypic and the compositional assessment. The GM hybrid to assess seed germination and pollen characteristics was produced crossing the transformed inbred line LH244 with the non-GM inbred line LH287. For the agronomic and phenotypic characteristics the non-GM hybrid NL6169, obtained crossing lines LH244 and HCL645, was used as the comparator in field trials (Table 1). While for seed germination and pollen characteristics the non-GM hybrid MPA640B, obtained crossing lines LH244 and LH287, was used as the comparator. The EFSA GMO Panel considers that NL6169 and MPA640B non-transgenic hybrid maize are appropriate conventional counterparts for the corresponding studies.

3.3.2. Statistical analysis of field trial data

The statistical analysis of the agronomic/phenotypic and compositional data from the 2011–2012 field trial study followed the recommendations of the GMO Panel (EFSA GMO Panel, 2010b, 2011a). This includes the application of a difference test (between the GM maize and its conventional counterpart) and an equivalence test (between the GM maize and the set of non-GM maize reference varieties). The results of the equivalence test are categorised into four possible outcomes (I-IV, ranging from equivalence to non-equivalence).

[25] The sites in 2011–2012 field trials were in Berdier, Gahan, Hunter, Los Indios, Sarasa, and in three in Pergamino. The additional site used to collect only agronomic and phenotypic characteristics was located in San Pedro. All the locations were in the Buenos Aires province.
[26] Additional information 22/3/2016.
[27] Additional information: 27/6/2016, 11/7/2016.
[28] The non-GM reference varieties were ACA 2002, ACA 430, Burrus 645 (used only in the agronomic and phenotypic analysis), Dekalb DK747, Dow AgroSciences Mill 527, Gateway 6116, H-9180, La Tijereta LT 625, Legacy L7671, LG2597, Midland Phillips 7B15P, Mycogen 2M746, NC+ 4443, Nidera AX 878, Phillips 717, Pioneer 33T56, Pioneer P1979, Stewart S518, Stewart S602, Stine 9724, Syngenta NK 880, Syngenta NK 910 and Syngenta NK 940.

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3.3.3. Agronomic and phenotypic analysis[29]

3.3.3.1. Agronomic and phenotypic characteristics tested under field conditions

Thirteen[30] agronomic and phenotypic endpoints, plus abiotic stressors, disease incidence and arthropod damage were collected from nine sites (Table 2). All endpoints were recorded appropriately at all sites.

The endpoint dropped ear count was not categorised because the estimate of the variance among reference varieties was 0; however, no significant differences are identified for this endpoint.

The test of difference and the test of equivalence could be applied to the remaining 12 endpoints, with the following results:

- For maize MON 87411 (treated with conventional herbicides), the test of difference identified statistically significant differences with the conventional counterpart for days to 50% pollen shed and 50% days to silking. The values of the four endpoints fall under equivalence category I.
- For maize MON 87411 (treated with intended herbicide), no statistically significant differences with the conventional counterpart are identified.

3.3.3.2. Agronomic and phenotypic characteristics tested under controlled conditions

Seed characteristcs[31]

Seed germination of maize MON 87411 F_2 grains from three different localities in the US[32] was compared with that of its conventional counterpart and with non-GM reference varieties[33] (Table 2). Grains were tested following a standardised assay[34] accepted by the Association of Official Seed Analysts (AOSA), and were, in addition, tested at six temperature regimes,[35] under controlled conditions. The across site analysis reveals no significant differences in the germination rate of maize MON 87411 F_2 grains compared to that of its conventional counterpart. Five significant differences in the germination characteristics are detected in the per-site analysis between maize MON 87411 and the conventional counterpart; however, the differences are not consistently detected across temperature regimes or across the three localities.

Although the applicant referred to seed dormancy when discussing the generated data on maize MON 87411 grain characteristics, no data on induced seed dormancy were supplied. The GMO Panel considers that only the conclusions on germination of maize MON 87411 F_2 grain are substantiated by the provided data.

Pollen characteristics[31]

The applicant reported data on pollen grain diameter and results of Alexander staining of pollen of MON 87411, its conventional counterpart and four commercial non-GM reference maize varieties[36] grown under field conditions in the US (Table 2). No significant differences between maize MON 87411 and its conventional counterpart are observed in pollen diameter and stain uptake; the latter is used to measure pollen viability.

3.3.4. Compositional analysis

For maize MON 87411, forage and grain harvested from the field trial study in Argentina in 2011–2012 (Table 2) were analysed for 78 constituents (9 in forage and 69 in grain), including the key constituents recommended by OECD (OECD, 2002). For 16 grain constituents,[37] more than 50% of the observations are below the limit of quantification. The statistical analysis was applied to the remaining 62 constituents

[29] Dossier: Part II – Section 1.3.5; Additional information 27/6/2016, 11/7/2016.
[30] Early stand count, days to 50% pollen shed, days to 50% silking, stay green, ear height, plant height, dropped ear count, stalk lodged plants, root lodged plants, final stand count, grain moisture, test weight, yield.
[31] Dossier: Part II – Section 1.3.5.4.
[32] Jefferson County, Iowa; Pawnee County, Kansas and Warren County Illinois.
[33] The non-GM reference varieties were Legacy L7671, Lewis 7007, Gateway 6116, Phillips 717, Midland Phillips 799, NC+ 5220, LG2540, Gateway 4148, H-9180, Stewart S588, LG2548.
[34] The assays consists in alternating 20°C for 16 h and 30°C for 8 h.
[35] The regimes were constant temperature at 5°C, 10°C, 20°C, 30°C, alternating 10°C and 20°C, and alternating 10°C and 30°C.
[36] Gateway 6158, Mycogen 2M746, LG2597 and Phillips 713.
[37] Sodium, furfural, and the fatty acids caprylic (8:0), capric (10:0), lauric (12:0), myristic (14:0), myristoleic (14:1), pentadecanoic (15:0), pentadecenoic (15:1), palmitoleic (16:1), heptadecanoic (17:0), heptadecenoic (17:1), γ-linolenic (18:3), eicosadienoic (20:2), eicosatrienoic (20:3) and arachidonic (20:4).

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(9 in forage[38] and 53 in grain[39]). A summary of the outcome of the test of difference and the test of equivalence is presented in Table 3:

- For maize MON 87411/not treated, statistically significant differences with its conventional counterpart were identified for 16 grain endpoints and 3 forage endpoints. All these endpoints fell under equivalence category I except for relative levels of palmitic acid which fell under equivalence category IV.
- For maize MON 87411/treated, statistically significant differences with its conventional counterpart are identified for 28 grain endpoints and 3 forage endpoints. All these endpoints fall under equivalence category I. Palmitic acid falls under equivalence category IV, although no statistically significant differences are identified with its conventional counterpart.

Table 3: Outcome of the comparative compositional analysis in grains and forage for maize MON 87411. The table shows the number of endpoints in each category

		Test of difference[(a)]			
		Not-Treated[(c)]		Treated[(c)]	
		Not different	Significantly different	Not different	Significantly different
Test of equivalence[(b)]	Category I/II	42	18[(d)]	29	31[(d)]
	Category III/IV	–	1[(e)]	1[(f)]	–
	Not categorised	1[(g)]	–	1[(g)]	–
	Total endpoints	62			62

(a): Comparison between maize MON 87411 and its conventional counterpart.
(b): Four different outcomes: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence). Not categorised means that the test of equivalence was not applied because of the lack of variation among the non-GM reference varieties.
(c): Treated/not-treated with intended herbicide: glyphosate (see Section 3.3.1).
(d): Endpoints with significant differences between maize MON 87411 and its conventional counterpart falling in equivalence category I-II (treated and not treated).
For grain, both treated and not treated: protein, aspartic acid, isoleucine, lysine, serine, threonine, eicosenoic acid, niacin, vitamin B1, carbohydrates by calculation, calcium, copper, iron, zinc, ash. For treated only: alanine, arginine, glutamic acid, histidine, leucine, phenylalanine, tyrosine, valine, stearic acid, vitamin B2, ADF, NDF, manganese, ferulic acid, calcium, moisture. For not treated only: oleic acid, linoleic acid, total fat.
For forage, both treated and not treated: calcium, ash. For treated only: moisture. For not treated only: carbohydrates by calculation.
(e): Endpoints with significant differences between maize MON 87411 and its conventional counterpart and falling in equivalence category III-IV. Estimated means are reported for these endpoints in Table 4.
(f): Palmitic acid in grain fell under equivalence category IV, although no statistically significant differences were identified with respect to its conventional counterpart.
(g): Endpoints not categorised for equivalence and with no significant differences between maize MON 87411 and its conventional counterpart: total fat (for forage in both not-treated and treated maize).

The GMO Panel assessed all significant differences between maize MON 87411 and its conventional counterpart, taking into account the potential impact on plant metabolism and the natural variability observed for the set of non-GM commercial reference varieties. Mean estimates for the endpoints showing significant differences between maize MON 87411 and its conventional counterpart and falling under category III/IV are given in Table 4.

[38] Ash, moisture, total fat, carbohydrates by calculation, protein, acid detergent fibre (ADF), neutral detergent fibre (NDF), calcium and phosphorus.
[39] Proximates (ash, moisture, protein, total fat and carbohydrates by calculation), fibre fractions (acid detergent fibre (ADF), neutral detergent fibre (NDF) and total detergent fibre (TDF)), amino acids (alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine), fatty acids (palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), arachidic acid (20:0), eicosenoic acid (20:1) and behenic acid (22:0)), vitamins (folic acid, β-carotene, thiamin, riboflavin, niacin, pyridoxine and α-tocopherol), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium and zinc) and other compounds (raffinose, ferulic acid, *p*-coumaric acid and phytic acid).

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Table 4: Quantitative results (estimated means and equivalence limits) for the endpoint with significant differences between maize MON 87411 and its conventional counterpart and falling under category IV in the test of equivalence (see Table 3)

	Endpoint (% total FA)	Maize MON 87411		Conventional counterpart	Non-GM reference varieties	
		Not treated	Treated[(a)]		Mean	Equivalence limits
Grain	Palmitic acid	13.68*	13.61	13.62[(b)]	11.32	9.64–12.99

For maize MON 87411, significantly different values are marked with an asterisk. FA = fatty acids.
(a): Treated with herbicide glyphosate as described in Section 3.3.1.
(b): Measured values for palmitic acid in the conventional counterpart were out of the equivalence limits derived from the selected non-GM reference varieties.

3.3.5. Conclusion on comparative analysis

None of the differences identified in the agronomic and phenotypic characteristics tested between maize MON 87411 and the conventional counterpart needs further assessment regarding potential environmental impact. The GMO Panel also concludes that none of the differences identified in forage and grain composition between maize MON 87411, its conventional counterpart and the non-GM commercial reference varieties needs further assessment regarding food and feed safety, except for palmitic acid levels in grains from not treated maize MON 87411, which are further assessed in Section 3.4.3.3.

3.4. Food and feed safety assessment

3.4.1. Effects of processing

Maize MON 87411 will undergo existing production processes used for conventional maize. No novel production process is envisaged. Therefore, processing of maize MON 87411 into food and feed products is not expected to result in products being different from those derived from non-GM varieties.

3.4.2. Influence of temperature and pH on newly expressed proteins

Effects of temperature and pH on CP4 EPSPS and Cry3Bb1 proteins have been previously evaluated by the EFSA GMO Panel (EFSA GMO Panel, 2009, 2015b, 2015c, 2017a,b). No new studies were provided in the context of this application.

3.4.3. Toxicology

3.4.3.1. Testing of the newly expressed proteins

The two proteins (CP4 EPSPS and Cry3Bb1) newly expressed in maize MON 87411 have been extensively characterised (Section 3.2.3).

The CP4 EPSPS protein has been previously assessed by the GMO Panel (e.g. EFSA GMO Panel 2009, 2011c) and no safety concerns for humans and animals were identified. Updated bioinformatics analysis did not reveal similarities of the CP4 EPSPS protein to known toxins. The GMO Panel is not aware of any new information that would change the previous conclusion of the risk assessment that the CP4 EPSPS protein does not raise safety concerns.

The Cry3Bb1 protein has been previously assessed by the GMO Panel (e.g. EFSA GMO Panel 2009, 2011c) and no safety concerns for humans and animals were identified. Updated bioinformatics analysis did not reveal similarities of the Cry3Bb1 protein to known toxins causing toxicity to humans and animals (except for Coleoptera). The GMO Panel is not aware of any new information that would change the previous conclusion of the risk assessment that the Cry3Bb1 protein does not raise safety concerns.

Based on scientific knowledge, no synergistic or antagonistic interactions exist between the two proteins newly expressed in maize MON 87411 which could raise safety concerns for food and feed are expected.

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Acute oral toxicity testing[40]

A bacterial Cry3Bb1 protein was administered at the dose of 5,000 mg/kg bw to male and female Crl:CD1(ICR) mice. No adverse effects related to the Cry3Bb1 protein were observed.

3.4.3.2. Testing of new constituents other than newly expressed proteins

No new constituents other than the newly expressed proteins have been identified in maize MON 87411, with the exception of the intended expression of DvSnf7 dsRNA and derived siRNAs, designed to control coleopteran pests via RNAi. According to the applicant, the gene-silencing effects of DvSnf7 dsRNA are mainly driven by ingestion of dsRNA from the plant and its processing into siRNAs by the insects.

NcRNAs are ubiquitous in the broad range of organisms used for food and feed and, hence, are normal constituents of human and animal diet. Dietary ncRNAs are generally rapidly denaturated, depurinated and degraded shortly after ingestion due to enzymes and conditions (e.g. pH) in the gastrointestinal tract lumen; in addition, the presence of barriers (e.g. mucus, cellular membranes) limits the cellular uptake of ncRNAs by gastrointestinal cells, and a rapid intracellular degradation of possible uptaken ncRNA occurs. Due to the above, the amount of RNAs taken up and absorbed after oral ingestion is considered negligible in humans and animals (mammals, birds and fish).

Moreover, the GMO Panel noted that the structure of DvSnf7 dsRNA does not show specific chemical modifications that would increase its stability in plant and/or its stability and cellular uptake in the gastrointestinal tract following oral administration.[41] Specifically, the applicant reported that DvSnf7 dsRNA has a typical hairpin structure, and does not contain any other structural modification aimed to increase stability.

Therefore, it is highly unlikely that the DvSnf7 dsRNA and its derived siRNAs are able to exert any biological effects once ingested by humans, mammals, birds and fish. Taking into account all of the above, the GMO Panel considers that no toxicological studies are necessary.

Nonetheless, the applicant provided a 28-day oral repeated-dose study in mice with DvSnf7 dsRNA,[26] conducted in accordance with OECD TG 407 and the principles of good laboratory practice (GLP). However, several deviations from OECD TG 407 requirements were observed. Therefore, the GMO Panel could not derive any conclusions from the study.

3.4.3.3. Information on altered levels of food and feed constituents

Palmitic acid is one of the most common saturated fatty acids found in oils and fats of animal origin used in human and animal diets. Palmitic acid is also endogenously synthesised in human and animal body from other fatty acids, carbohydrates and amino acids (Carta et al., 2017).

The minimal increase in the level of palmitic acid in maize MON 87411 as compared to the conventional counterpart is considered of no toxicological concern by the GMO Panel.

3.4.3.4. Testing of the whole genetically modified food and feed

No substantial modifications in the composition of maize MON 87411, no indication of possible unintended effects relevant for food/feed safety were identified. Therefore, animal studies on the food/feed derived from maize MON 87411 were not necessary (EFSA GMO Panel, 2011a). In accordance with Regulation (EU) No 503/2013, the applicant provided a 90-day oral repeated-dose toxicity study on whole food and feed from maize MON 87411 in rats. Animal feeding studies in broilers and channel catfish fed diets containing maize MON 87411 material were also provided according to Regulation (EU) No 503/2013. All these studies were evaluated by the EFSA GMO Panel.

90-day feeding study in rat

Pair-housed Crl:CD(SD) rats (16 per sex per group) were allocated to two groups using a randomized complete block design with eight replications. Groups were fed test or control diets containing approximately 33% (w/w) ground grain from MON 87411 sprayed with glyphosate-containing herbicide (test item) or from the conventional counterpart NL6169 (control material) respectively. The study provided was adapted from OECD TG 408 and complying with the principles of GLP.

Event-specific PCR analysis on grains confirmed the molecular identity of MON 87411 grains. Both test item and control materials were analysed for proximates, amino acids, minerals, mycotoxins and pesticides.

[40] Additional Information: 5/10/2017.
[41] Additional information 30/5/2017.

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Balanced diets were prepared according to the specifications for PMI Certified Rodent LabDiet #5002, with ground grain inclusion rate of 33% (w/w).

The stability of the test and control materials was not tested; however, in accordance with product expiration standards declared by the diet manufacturer, the constituents of the diets are considered stable for the duration of the treatment. Currently there are no practical analytical methods available to determine homogeneity and concentration of ground grains in the formulated diets. Diet preparation procedures and regular evaluations of the mixing methods by surrogate analytes guarantee their homogeneity and the proper concentration of the test or control substances in them.

Feed and water were provided *ad libitum*. Animals were checked twice daily for mortality and clinical signs. Detailed physical examinations were conducted on all animals pre-treatment, then weekly during the dosing period and on the day of the scheduled necropsy. Individual body weights were recorded pre-treatment and then weekly during the dosing period and on the day prior to the scheduled necropsy. Feed consumption (per cage) was determined weekly during the study. Ophthalmoscopy and functional observation battery (FOB) data and motor activity were recorded on all animals pre-treatment and at the end of the study (week 12). Clinical pathology (i.e. haematology, clinical chemistry and coagulation, urine analyses) and necropsy examination with selected organs weighing were conducted at the end of the treatment period on all animals. The animals were fasted overnight prior to blood collection while in metabolism cages for urine collection. Organs and tissues from all sacrificed animals as well as gross lesions were subjected to a detailed histopathological examination. Upon completion of the histopathologic assessment of all tissues, histopathology was reviewed by a peer review pathologist.

Mean, median, standard deviation, min and max were reported for all continuous endpoints for each group/sex and per period or time as appropriate. The applicant performed a power analysis per gender, using a pre-specified effect sizes[42] for eight endpoints[43] with a 5% level of significance.

For all selected endpoints the power estimates were greater than 99%, with the exception of absolute lymphocytes (80% for males and 60% for females), and body weight (80% for males and 88% for females).

The cage or the individual animal was considered the experimental unit according to the corresponding estimate of the cage effect.[44] The in-life and terminal body weights/gain, organ weights, feed consumption/efficiency, clinical pathology and functional observations, when appropriate, were checked for homogeneity and normality,[45] analysed with ANOVA and tested using a t-test. Finally, outcome proportions of incidence of functional observations were analysed with the Fisher's exact test. In response to a request from EFSA, the difference between test and control groups and associated 95% confidence interval were also presented in terms of standardised effect size (i.e., normalised to standard deviation[46]). The goodness of fit was evaluated by visual examination of residual plots and histograms.[47] Based on this evaluation, the models were considered appropriate.

No mortality was observed during the study. No test diet-related clinical signs and ophthalmoscopic findings were seen.

Statistically significant lower mean feed consumption (as g/cage per day only) were observed in males fed test diet (~ 9% in study week intervals 5–6, 9–10, 10–11, 11–12). This was associated with a statistically significant decrease in mean body weights, compared to the concurrent control (~ 7% in weeks 11 and 12) and in mean cumulative body weight (~ 12% in study week intervals 0–10, 0–11 and 0–12). Moreover, statistically significant lower mean weekly body weight change was also observed in males (study week intervals 0–1, 3–4, and 6–7) and in females (study week interval 7–8) fed the test diet, compared to the concurrent controls. In the absence of test diet-related clinical signs and histopathological changes in the digestive tract, the GMO Panel considers that these changes are not adverse.

No statistically significant differences in FOB and locomotor activity parameters were observed between animals fed the test and control diets at the end of the study, with the exception of lower

[42] Defined on the basis of six previous studies: WIL-50283, WIL-50296, WIL-50297, WIL-50333, WIL-50342, and WIL-50370 and additional information 20/9/2016.
[43] Eight endpoints were selected: absolute lymphocytes, alkaline phosphatase, body weight, cholesterol, creatinine, urea nitrogen, kidney weight, liver weight.
[44] Additional information received on 22/9/2016 and 10/1/2017.
[45] Normality and heterogeneity assumptions were checked by visual examination of residual plots and histogram. No extreme violations of the assumptions were observed.
[46] Additional information 10/1/2017.
[47] Additional information 15/6/2017.

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mean grooming observation periods in females fed the test diet. Similar observations in females were reported during the pre-test. The GMO Panel considers this isolated finding not to be treatment-related.

Statistically significant lower mean corpuscular haemoglobin (MCH) (~ 3%) was observed in males fed the test diet, compared to the concurrent control. The GMO Panel considers this isolated finding not to be treatment related. No statistically significant differences in coagulation parameters (PT and APTT) were observed in animals fed the test diet, compared to concurrent control.

Statistically significant lower mean glucose (~ 6%) and mean triglycerides (~ 14%) were observed in females fed the test diet, compared to concurrent control. The GMO Panel considers these small changes not adverse.

No statistically significant differences in urinalysis parameters were observed in animals fed the test diet, compared to concurrent control.

No statistically significant differences in organ weights were observed in animals fed the test diet, compared to concurrent control, except for a higher relative mean testes weight (~ 10%) in males fed the test diet. This increase was not associated with histopathological changes in the testes histopathology, and therefore not considered adverse.

No treatment-related gross lesions or microscopic findings were noted in organs or tissues. Sporadic histopathological findings are considered compatible with the spontaneous background pathology of rats of this strain and age.

The GMO Panel concluded that no maize MON 87411-related adverse effects were observed in this study.

The GMO Panel noted that the applicant only tested one dose level. However, the dose tested was close to the highest possible without inducing nutritional imbalance according to the current knowledge, and in accordance with the limit test dose as described in OECD TG 408. Therefore, this is not considered to affect the above conclusions.

42-day broiler study[26]

A total of 700 (350 per sex) one-day-old chicken broilers (Cobb x Cobb 500) were randomly allocated to seven dietary groups with 100 chicks per treatment (10 pens per treatment, half for each sex, ten birds per pen) and fed balanced diets[48] containing up to 57% maize grain from maize MON 87411 (test item), the conventional counterpart NL6169 (control item) or one of the five commercial varieties ACA430, Mycogen 2M746, La Tijereta LT625, Daw AgroSciences Mill 527 and Burrus 645 (reference items). Diets (as crumbled pellets or pellets) and water were offered *ad libitum*. No significant differences between the groups fed test and control diets were observed in mortality (about 3%), final body weight, weight gain, feed to gain ratio, and yield of pre-chill organs and post-chilled carcass and cuttable part percentages.

The GMO Panel concludes that administration of diets containing up to 57% maize grain from maize MON 87411 to broilers did not cause adverse effects. Moreover, the measured performance endpoints were similar between groups fed balanced diets containing GM and non-GM maize (conventional counterpart and references).

8-week channel fish study[26]

A total of 600 channel catfishes (sex undetermined) were randomly allocated to six dietary groups with 100 catfishes per treatment (five aquaria per treatment, twenty fishes per aquaria) and fed balanced diets formulated as sinking pellets containing 30% ground maize grain from maize MON 87411, the conventional counterpart maize NL6169 or one of the four reference varieties ACA 430, Mycogen 2M746, Burrus 645, La Tijereta LT 625. No mortality and no abnormal behaviours were observed among fishes in any aquarium during the study. There were no statistically significant differences in overall weight gain per fish, total diet consumption per fish, or diet conversion ratio among fish fed the reference, control and test diets.

The GMO Panel concludes that administration of balanced diets containing up to 30% maize grain MON 87411 to channel catfishes, up to 8 weeks, did not cause adverse effects. Moreover, the measured performance endpoints were similar between groups fed balanced diets containing GM and non-GM maize (conventional counterpart and references).

[48] starter (0–21 days) and grower/finisher (22–42 days) diets.

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3.4.4. Allergenicity

The strategies to assess the potential risk of allergenicity focus on the source of the recombinant protein, on the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons and on whether the transformation may have altered the allergenic properties of the modified plant.

3.4.4.1. Assessment of allergenicity of the newly expressed proteins[49]

A weight-of-evidence approach was followed, taking into account all of the information obtained on the newly expressed protein, as no single piece of information or experimental method yield sufficient evidence to predict allergenicity (Codex Alimentarius, 2009; EFSA GMO Panel 2011a; Regulation (EU) 503/2013).

The *cry3Bb1* gene (encoding for the Cry3Bb1 protein) and the *cp4 epsps* gene (encoding for the CP4 EPSPS protein) originate from *B. thuringiensis* and *Agrobacterium* sp., respectively, which are not considered to be common allergenic sources.

Updated bioinformatics analyses of the amino acid sequences of the Cry3Bb1 and CP4 EPSPS proteins, using the criterion of 35% identity in a sliding window of 80 amino acids, revealed no significant similarities to known allergens. In addition, the applicant also performed analyses searching for matches of eight contiguous identical amino acid sequences between the Cry3Bb1 and CP4 EPSPS proteins and known allergens, which confirmed the outcome of the previous bioinformatics analysis.

The studies on resistance to degradation of the Cry3Bb1 and CP4 EPSPS proteins by pepsin have been previously assessed by the GMO Panel (EFSA GMO Panel, 2009, 2015c).

The GMO Panel has previously evaluated the safety of the Cry3Bb1 and CP4 EPSPS proteins in the context of other applications and no concerns on allergenicity were identified (e.g. EFSA GMO Panel, 2009, 2011c).

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. Vazquez et al., 1999). The GMO Panel has previously evaluated the safety of the Cry3Bb1 protein and no concerns on adjuvanticity in the context of the applications assessed were identified (EFSA GMO Panel, 2009, 2011c). From the limited experimental evidence available, the GMO Panel did not find indications that the presence of the Bt protein at the levels expressed in maize MON 87411 might act as adjuvants with the potential to enhance a specific immunoglobulin E (IgE) response and to favour the development of an allergic reaction.

In the context of the present application, the GMO Panel considers that there are no indications that the newly expressed Cry3Bb1 and/or CP4 EPSPS proteins in maize MON 87411 may be allergenic.

3.4.4.2. Assessment of allergenicity of the whole GM plant or crop[50]

The GMO Panel regularly reviews the available publications on food allergy to maize. However, to date, maize has not been considered a common allergenic food[51] (OECD, 2002). Therefore, the GMO Panel did not request experimental data to analyse the allergen repertoire of GM maize.

The applicant provided spontaneous information where lipid transfer protein (LTP) expression levels in maize MON 87411 were compared to those in the conventional counterpart and commercial reference varieties. No changes in expression levels raising concern were identified.

3.4.5. Dietary exposure assessment to endogenous and new constituents

In line with Regulation (EU) No 503/2013, the applicant provided dietary exposure to Cry3Bb1 and CP4 EPSPS proteins present in maize MON 87411. In addition, the applicant provided a dietary exposure assessment for DvSnf7 dsRNA. However, as discussed in Section 3.2.4, the GMO Panel considers that the levels of dsRNA are not a good proxy for the levels of the active siRNAs in the plant. Moreover, the human and animal dietary exposure to DvSnf7 dsRNA and derived siRNA is

[49] Dossier: Part II – Section 1.5.1, 1.5.3, and additional information on 22/3/2016, 6/10/2017 and 20/12/2017.
[50] Dossier: Part II – Section 1.5.2 and additional information on 22/3/2016.
[51] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

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considered negligible since these molecules are generally rapidly denaturated, depurinated and degraded shortly after ingestion (see Section 3.4.3.4).

3.4.5.1. Human dietary exposure

Only acute dietary exposure estimates for the Cry3Bb1 and CP4 EPSPS proteins were provided by the applicant. Dietary exposure was estimated across different European countries on different population groups: young population (toddlers, other children) and adult population (adolescents, adults, elderly and very elderly).

For the purpose of estimating dietary exposure, the mean protein expression levels in treated grains from replicated field trials across five locations in Argentina (see Table 1) were used. Mean values of 3.5 and 1.7 μg/g (fresh weight) were used for Cry3Bb1 and CP4 EPSPS proteins respectively. Since no specific consumption data were available on the consumption of commodities containing maize MON 87411, a conservative scenario with 100% replacement of conventional maize was considered. Maize oil was excluded from the assessment since no proteins are expected to be present in the oil. Consumption data of the relevant commodities (corn bread, corn flakes, corn milling products, cornmeal porridge, corn grain, corn snacks, sweet corn and popcorn) were retrieved by the applicant from the available summary statistics of the EFSA Comprehensive European Food Consumption Database.[52] The EFSA consumption database contains information on food consumption data at individual level from the most recent national dietary surveys in different EU Member States (EFSA, 2011; Huybrechts et al., 2011; Merten et al., 2011).

Acute dietary exposure was estimated using for each population group the food commodity with the highest acute consumption among consumers only (95th or 97.5th percentile depending on the number of consumers),[53] and multiplying this value by the mean values of Cry3Bb1 and CP4 EPSPS proteins. The applicant used the mean values reported for the NEPs in grain also in the consumed processed foods; this adds uncertainty to the exposure estimations since the effect on processing factors or recipes is not considered. Among the young population, the highest acute exposure was estimated in toddlers (1–3 years) following the consumption of sweet corn (31.5 μg/kg bw per day and 15.3 μg/kg bw per day for Cry3Bb1 and CP4 EPSPS respectively), while for adults (18–65 years) the consumption of popcorn led to the highest acute exposure estimates (12.3 μg/kg bw per day and 6.0 μg/kg bw per day for Cry3Bb1 and CP4 EPSPS respectively). The use of the highest acute consumption for only one food commodity could slightly underestimate the dietary exposure to Cry3Bb1 and CP4 EPSPS proteins in certain population groups.

The GMO Panel estimated chronic dietary exposure to Cry3Bb1 and CP4 EPSPS proteins, which was not provided by the applicant. Individual consumption data on food commodities from dietary surveys with at least 2 days consumption and covering a total of 22 European countries[54] were retrieved from the EFSA Comprehensive European Food Consumption Database. Different recipes and processing factors were considered to estimate the amount of maize in the consumed commodities before assigning Cry3Bb1 and CP4 EPSPS protein levels to the relevant commodities.[55] No losses of NEPs during processing were considered. The highest chronic dietary exposure in the highly exposed population (95th percentile) was estimated in one dietary survey in infants (0–12 months) for both Cry3Bb1 (15.7 μg/kg bw per day) and CP4 EPSPS (7.6 μg/kg bw per day), while in the adult population the highest dietary exposure was estimated in adolescents (10–18 years) with values of 4.6 μg/kg bw per day for Cry3Bb1 and 2.2 μg/kg bw per day for CP4 EPSPS. Overall, sweet corn and corn flakes were the main contributors to the chronic dietary exposure to both NEPs.

3.4.5.2. Animal dietary exposure

Daily dietary exposure (DDE) to the Cry3Bb1 and CP4 EPSPS proteins newly expressed in maize MON 87411 was provided by the applicant across different livestock animal species (broiler, lactating dairy cow and finishing pig), based on estimates provided for the EU by OECD (2009), for animal body weight, daily feed intake and the inclusion rates (percentage) of maize grains, gluten feed and gluten

[52] https://www.efsa.europa.eu/en/applications/gmo/tools

[53] EFSA (European Food Safety Authority), 2011. Evaluation of the FoodEx, the food classification system applied to the development of the EFSA Comprehensive European Food Consumption Database. EFSA Journal 2011;9(3):1970, 27 pp. https://doi.org/10.2903/j.efsa.2011.1970

[54] Austria, Belgium, Bulgaria, Croatia, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Portugal, Romania, Spain, Sweden, the Czech Republic, the Netherlands and United Kingdom.

[55] Example: 100 g of corn bread contains 26.4 g of corn flour that are derived from 31.7 g of maize grains (processing factor of 1.22); this would result in 1.13 μg of Cry3Bb1/g of corn bread.

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meal in animal diets. A conservative scenario with 100% replacement of the conventional maize (grain, gluten feed and gluten meal) was considered. The mean and highest levels of Cry3Bb1 and CP4 EPSPS proteins in grains (Section 3.2.4, Table 1) were used to estimate the mean and highest protein levels in gluten feed and gluten meal, calculated to be 2.6 and 7.1-fold higher than in maize grain, based on the protein content of gluten feed and gluten meal relative to maize grain (OECD, 2002), assuming that no protein is lost during the processing.

Estimated DDEs to the Cry3Bb1, based only on mean levels in GM maize grain, gluten feed and gluten meal were respectively 197, 74 and 200 μg/kg per bw in broiler; 46, 120 and 217 μg/kg per bw in lactating dairy cow; 84, 62 and 84 μg/kg per bw in finishing pig.

Estimated DDEs to the CP4 EPSPS protein, based only on mean levels in GM maize grain, gluten feed and gluten meal were respectively 94, 35 and 96 μg/kg per bw in broiler; 22, 56 and 104 μg/kg per bw in lactating dairy cow; 160, 30 and 40 μg/kg per bw in finishing pig.

The GMO Panel estimated DDE to the Cry3Bb1 and CP4 EPSPS proteins across different livestock animal species (beef and dairy cows, lamb and breeding swine) based on estimates, as provided for the EU by OECD (2009), for animal body weight, daily feed intake and inclusion rates (percentages) of field maize forage/silage in animal diets (information not provided by the applicant). A conservative scenario with 100% replacement of conventional maize (forage/silage) by the GM maize was considered. Mean levels of the Cry3Bb1 and CP4 EPSPS proteins in forage (Section 3.2.4, Table 1) were used as occurrence data.

Estimated DDEs to the Cry3Bb1 protein, based on the consumption of GM maize forage/silage, were 748 μg/kg bw in beef, 897 μg/kg per bw in dairy cow, 497 μg/kg bw in lamb and 180 μg/kg bw in breeding swine.

Estimated DDEs to the CP4 EPSPS protein, based on the consumption of GM maize forage/silage, were 153 μg/kg bw in beef, 184 μg/kg bw in dairy cow, 102 μg/kg bw in lamb and 37 μg/kg bw in breeding swine.

3.4.6. Nutritional assessment of GM food and feed

The intended trait of maize MON 87411 is insect resistance and herbicide tolerance, with no intention to alter the nutritional parameters. However, palmitic acid levels in not-treated maize MON 87411 were significantly different from its conventional counterpart and showed a lack of equivalence with the set of non-genetically modified reference varieties (Section 3.3.4). The biological role of this compound, its levels in this maize and the magnitude and direction of the observed changes were considered in the nutritional assessment.

3.4.6.1. Human nutrition

Palmitic acid (16:0) is a saturated fatty acid present in the diet and synthesized endogenously, and an essential component of cell membranes, secretory and transport lipids, with crucial roles in protein palmitoylation and palmitoylated signal molecules (German, 2011). Palmitic acid is the main saturated fatty acid present in maize oil, although there are other foods such as meat, milk, and derived products that contain higher amounts of palmitic acid and are the main contributors to the dietary intake of this fatty acid. As shown in Section 3.3.4, in the non-treated maize MON 87411 there was an increase of 0.064% (% total FA) as compared to the conventional counterpart. After considering the extent of the increase and, above all, the limited role of maize and maize-based products as a source of palmitic acid in the human diet, the GMO Panel concludes that the nutritional impact of foods derived from the maize MON 87411 is similar to that expected from the conventional counterpart and non-GM commercial reference varieties.

3.4.6.2. Animal nutrition

Palmitic acid is a common saturated fatty acid found in oils and fats of vegetable and animal origin used in animal diets (Loften et al., 2014; Duran-Montgé et al., 2007, Tancharoenrat et al., 2014); it is also endogenously synthetised in animal body, i.e. milk palmitic acid arises both from diet and de novo synthesis (Palmquist, 2006). Feeding palmitic acid could affect fat fatty acid composition, i.e. feeding highly concentrated sources of palmitic acid to dairy cows increases significantly C16:0 in milk fat (Loften et al., 2014). Therefore, also considering the extent of the increase observed, the GMO Panel concludes that the nutritional impact of feeds derived from the maize MON 87411 is similar to that expected from the conventional counterpart and non-GM commercial reference varieties.

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3.4.7. Post-market monitoring of GM food/feed

The GMO Panel concludes that maize MON 87411, as described in this application, is nutritionally equivalent to and as safe as the conventional counterpart and the non-GM maize reference varieties tested, and no post-market monitoring (EFSA GMO Panel, 2011a) of food/feed is considered necessary.

3.4.8. Conclusion on the food/feed safety assessment

The GMO Panel did not identify safety concerns regarding the toxicity and allergenicity of the proteins CP4 EPSPS and Cry3Bb1 expressed in maize MON 87411 and found no evidence that the genetic modification might significantly change the overall allergenicity of maize MON 87411. Based on the information provided in the frame of this application with regard to DvSnf7 dsRNA and deriving siRNAs, the Panel considers that no safety concerns are associated with the presence of these compounds in maize MON 87411.

Based on the outcome of the comparative assessment and the nutritional assessment, the GMO Panel concludes that the nutritional impact of maize MON 87411-derived food and feed is expected to be the same as those derived from the conventional counterpart and non-GM commercial reference varieties. The GMO Panel concludes that maize MON 87411 is nutritionally equivalent to and as safe as the conventional counterpart and the non-GM reference varieties tested.

3.5. Environmental risk assessment and monitoring plan

Considering the scope of application EFSA-GMO-NL-2015-124, which excludes cultivation, the ERA of maize MON 87411 mainly takes into account: (1) the exposure of microorganisms to recombinant DNA in the gastrointestinal tract of animals fed GM material and of microorganisms present in environments exposed to faecal material of these animals (manure and faeces); and (2) the accidental release into the environment of viable maize MON 87411 grains during transportation and processing (EFSA GMO Panel, 2010a).

3.5.1. Environmental risk assessment[56]

3.5.1.1. Persistence and invasiveness of the GM plant[31]

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

It is unlikely that the intended traits of maize MON 87411 will provide a selective advantage to maize plants, except when they are exposed to glyphosate-containing herbicides or infested by insect pests that are susceptible to the *DvSnf7* dsRNA or to the Cry3Bb1 protein. However, this fitness advantage will not allow the GM plant to overcome other biological and abiotic factors (described above) limiting the persistence and invasiveness of the plant. Therefore, the presence of the intended traits will not affect the persistence and invasiveness of the GM plant.

In conclusion, the GMO Panel considers it very unlikely that maize MON 87411 will differ from conventional maize hybrid varieties in its ability to survive until subsequent seasons, or to establish occasional feral plants under European environmental conditions in case of accidental release into the environment of viable maize MON 87411 grains.

3.5.1.2. Potential for gene transfer

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

[56] Dossier: Part II – Section 5.

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Plant-to-microorganism gene transfer[57]

Genomic DNA can be a component of food and feed products derived from maize. It is well documented that such DNA becomes substantially degraded during processing and digestion in the human or animal gastrointestinal tract. However, bacteria in the digestive tract of humans and animals, and in other environments, may be exposed to fragments of DNA, including the recombinant fraction of such DNA.

Current scientific knowledge of recombination processes in bacteria suggests that horizontal transfer of non-mobile, chromosomally located DNA fragments between unrelated organisms (such as from plants to bacteria) is not likely to occur at detectable frequencies under natural conditions (for further details, see EFSA, 2009).

The only mechanism known to facilitate horizontal transfer of non-mobile, chromosomal DNA fragments to bacterial genomes is HR. This requires the presence of at least two stretches of DNA sequences that are similar in the recombining DNA molecules. In the case of sequence identity with the transgene itself, recombination would result in gene replacement. In the case of identity with two or more regions flanking recombinant DNA, recombination could result in the insertion of additional DNA sequences in bacteria and thus confer the potential for new properties.

Maize MON 87411 contains genetic elements of bacterial origin. These are: (1) the *cry3Bb1* (codon-optimised coding sequence for the Cry3Bb1 protein from *B. thuringiensis*); (2) the *cp4 epsps* (codon-optimised coding sequence for CP4 EPSPS protein from *Agrobacterium* sp. strain CP4); and (3) the left and right borders form *A. tumefaciens* used for the T-DNA transfer.

Bioinformatic analyses of the inserted DNA demonstrated that the bacterial genes *cry3Bb1*, and *cp4 epsps* did not provide sufficient sequence identity to facilitate HR. A sufficient sequence identity with bacterial DNA was found only for the left border from *A. tumefaciens*; therefore a double HR of transgenic plant DNA with plasmid DNA of microorganisms is unlikely.

In summary, there is no indication for an increased likelihood of HGT of DNA from maize MON 87411 to bacteria. Given the nature of the recombinant DNA, the GMO Panel identified no safety concern linked to an unlikely but theoretically possible HGT.

Plant-to-plant gene transfer[33]

The potential for occasional feral GM maize MON 87411 plants originating from grain import spills to transfer recombinant DNA to sexually compatible plants and the environmental consequences of this transfer were considered.

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

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

The potential of spilled maize grains to establish, grow and produce pollen is extremely low and transient (see Section 3.5.1.1). Therefore, the likelihood/frequency of cross-pollination between occasional feral GM maize plants resulting from grain spillage, and weedy or cultivated *Zea* plants is considered extremely low (EFSA, 2016). Even if cross-pollination would occur, the GMO Panel is of the opinion that environmental effects as a consequence of the spread of genes from occasional feral GM maize plants in Europe will not differ from that of conventional maize varieties.

3.5.1.3. Interactions of the GM plant with target organisms[58]

Taking the scope of the application EFSA-GMO-NL-2015-124 into account (no cultivation), potential interactions of occasional feral maize MON 87411 plants arising from grain import spills with the target organisms are not considered a relevant issue.

[57] Dossier: Part II – Section 5.3.2; Additional information: 6/10/2017.
[58] Dossier: Part II – Section 5.3.3.

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3.5.1.4. Interactions of the GM plant with non-target organisms[59]

Given that environmental exposure of non-target organisms to spilled GM grains or occasional feral GM maize plants arising from spilled maize MON 8711 grains is limited and because ingested dsRNA and proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions of the maize MON 87411 with non-target organisms are not considered to raise any environmental safety concern.

3.5.1.5. Interactions with the abiotic environment and biogeochemical cycles[60]

Given that environmental exposure to spilled grains or occasional feral maize MON 87411 plants arising from grain import spills is limited and because ingested dsRNA and proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions with the abiotic environment and biogeochemical cycles are not considered to raise any environmental safety concern.

3.5.2. Post-market environmental monitoring[61]

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) identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment that were not anticipated in the ERA.

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

As the ERA did not identify potential adverse environmental effects from maize MON 87411, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for maize MON 87411 includes: (1) the description of a monitoring approach involving operators (federations involved in import and processing), reporting to the applicant, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment; (2) a coordinating system established by EuropaBio for the collection of information recorded by the various operators; and (3) the review of relevant scientific publications retrieved from literature searches (Lecoq et al., 2007; Windels et al., 2008). The applicant proposes to submit a PMEM report on an annual basis and a final report at the end of the authorisation period.

The GMO Panel considers that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of maize MON 87411. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

In the context of PMEM, the applicant should improve the literature searches according to the GMO Panel recommendations given in Section 3.1.

3.5.3. Conclusion on the environmental risk assessment and monitoring plan

The GMO Panel concludes that it is unlikely that maize MON 87411 would differ from conventional maize varieties in its ability to persist under EU environmental conditions. Considering the scope of the application EFSA-GMO-NL-2015-124, interactions of occasional feral maize MON 87411 plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from maize MON 87411 to bacteria does not indicate a safety concern. Therefore, considering the introduced traits, the outcome of the comparative analysis, the routes and levels of exposure, the GMO Panel concludes that maize MON 87411 would not raise safety concerns in the event of accidental release of viable GM maize grains into the environment.

The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of maize MON 87411.

3.6. Conclusions

The GMO Panel was asked to carry out a scientific assessment of maize MON 87411 for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003.

[59] Dossier: Part II – Section 5.3.4
[60] Dossier: Part II – Section 5.3.6
[61] Dossier: Part II – Section 6.

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The molecular characterisation data establish that maize MON 87411 contains a single insert consisting of one copy of the *cp4 epsps* and the *cry3Bb1* expression cassettes and one copy of the DvSnf7 dsRNA expression cassette. Bioinformatics analyses of the sequences encoding the newly expressed proteins and other ORFs within the insert, or spanning the junctions between the insert and genomic DNA, do not indicate significant similarities to toxins and allergens. The in planta RNAi off-target search, performed with the sequence of the DvSnf7 dsRNA, does not provide indication for an off-target effect that would require further safety assessment. The stability of the inserted DNA and of the introduced trait was confirmed over several generations. The methodology used to quantify the levels of the CP4 EPSPS and Cry3Bb1 proteins is considered adequate. The protein characterisation data comparing the structural and biochemical properties of plant- and microbe-derived CP4 EPSPS and Cry3Bb1 proteins indicate that these proteins are equivalent and the microbe-produced protein can be used in the safety studies.

The GMO Panel considers that none of the agronomic and phenotypic differences between maize MON 87411 and the conventional counterpart needed further assessment, given the magnitude of the observed differences, the nature of the endpoints and the outcome of the equivalence test.

The GMO Panel also concludes that none of the differences identified in forage and grain composition between maize MON 87411, its conventional counterpart, and the non-GM commercial reference varieties needs further assessment regarding food and feed safety, except for palmitic acid levels in grains from not-treated maize MON 87411, which were further assessed.

The GMO Panel did not identify safety concerns regarding the toxicity and allergenicity of the CP4 EPSPS and Cry3Bb1 proteins expressed in maize MON 87411 and found no evidence that the genetic modification might significantly change the overall allergenicity of maize MON 87411. The nutritional impact of maize MON 87411-derived food and feed is expected to be the same as those derived from the conventional counterpart and non-GM commercial reference varieties. The GMO Panel concludes that maize MON 87411, as described in this application, is nutritionally equivalent to and as safe as the conventional counterpart and the non-GM maize reference varieties tested, and no post-market monitoring of food/feed is considered necessary.

The GMO Panel concludes that there is a very low likelihood of environmental effects resulting from the accidental release of viable grains from maize MON 87411 into the environment. The PMEM plan and reporting intervals are in line with the intended uses of maize MON 87411.

Based on the relevant publication identified through the literature searches, the GMO Panel did not identify any safety issues pertaining to the intended uses of maize MON 87411. In the context of PMEM, the applicant should improve future literature searches according to the GMO Panel recommendations.

In conclusion, the GMO Panel considers that maize MON 87411, as described in this application, is as safe as its conventional counterpart and the tested non-GM maize reference varieties with respect to potential effects on human and animal health and the environment.

Documentation provided to EFSA

- Letter from the Competent Authority of the Netherlands received on 10 February 2015 concerning a request for placing on the market of genetically modified maize MON 87411 submitted by Monsanto Europe S.A./N.V. in accordance with Regulation (EC) No 1829/2003 (application reference EFSA-GMO-NL-2015-124).
- Acknowledgement letter dated 12 February 2015 from EFSA to the Competent Authority of the Netherlands.
- Letter from EFSA to applicant dated 25 March 2015 requesting additional information under completeness check.
- Letter from applicant to EFSA received on 24 April 2015 providing additional information under completeness check.
- Letter from EFSA to applicant dated 27 May 2015 requesting additional information under completeness check.
- Letter from applicant to EFSA received on 27 July 2015 providing additional information under completeness check.
- Letter from EFSA to applicant dated 17 August 2015 delivering the 'Statement of Validity' of application EFSA-GMO-NL-2015-124 for the placing on the market of maize MON 87411 submitted by Monsanto Europe S.A./N.V. in accordance with Regulation (EC) No 1829/2003.

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- Letter from EFSA to applicant dated 8 September 2015 requesting additional information and stopping the clock.
- Letter from applicant to EFSA received on 14 September 2015 providing additional information.
- Letter from EFSA to applicant dated 10 November 2015 requesting additional information and maintaining the clock stopped.
- Letter from applicant to EFSA received on 20 November 2015 providing additional information.
- Letter from EFSA to applicant dated 21 December 2015 requesting additional information and maintaining the clock stopped.
- Letter from EFSA to applicant dated 11 February 2016 requesting additional information and maintaining the clock stopped.
- Letter from applicant to EFSA received on 22 March 2016 providing additional information.
- Letter from applicant to EFSA received on 3 May 2016 providing additional information.
- Email from EFSA to applicant dated 10 May 2016 re-starting the clock as of 3 May 2016.
- Letter from EFSA to applicant dated 13 May 2016 requesting additional information and stopping the clock.
- Letter from applicant to EFSA received on 17 May 2016 requesting clarifications.
- Letter from EFSA to applicant dated 23 May 2016 providing clarifications concerning requested.
- Letter from applicant to EFSA received on 27 June 2016 providing additional information.
- Email from EFSA to applicant dated 30 June 2016 re-starting the clock as of 27 June 2016.
- Letter from EFSA to applicant dated 1 July 2016 requesting additional information and stopping the clock.
- Letter from applicant to EFSA received on 11 July 2016 providing additional information.
- Email from EFSA to applicant dated 11 July 2016 re-starting the clock as of 11 July 2016.
- Letter from EFSA to applicant dated 20 July 2016 requesting additional information and stopping the clock.
- Letter from applicant to EFSA received on 16 September 2016 providing amended already-submitted information.
- Letter from applicant to EFSA received on 22 September 2016 providing additional information.
- Email from EFSA to applicant dated 23 September 2016 re-starting the clock as of 22 September 2016.
- Letter from EFSA to applicant dated 26 September 2016 requesting additional information and stopping the clock.
- Letter from applicant to EFSA received on 24 November 2016 providing additional information.
- Email from EFSA to applicant dated 25 November 2016 re-starting the clock as of 24 November 2016.
- Letter from EFSA to applicant dated 5 December 2016 requesting additional information and stopping the clock.
- Letter from applicant to EFSA received on 10 January 2017 providing additional information.
- Email from EFSA to applicant dated 12 January 2017 re-starting the clock as of 10 January 2017.
- Email from EFSA to applicant dated 13 January 2017 providing clarifications on the risk assessment of the application.
- Letter from EFSA to applicant dated 19 January 2017 requesting additional information and stopping the clock.
- Letter from applicant to EFSA received on 16 February 2017 requesting an extended timeline for submission of responses.
- Letter from EFSA to applicant dated 22 February 2017 requesting clarifications on the rationale behind the request to extend the timeline for submission of responses.
- Letter from applicant to EFSA received on 7 March 2017 providing justifications for the request to extend the timeline for submission of responses.
- E-mail from EFSA to applicant dated 13 March 2017 accepting the request to extend the timeline for submission of responses as indicated in EFSA letter dated 19 January 2017.
- Letter from EFSA to applicant dated 29 March 2017 requesting additional information and maintaining the clock stopped.
- Letter from EFSA to applicant dated 3 May 2017 requesting additional information and maintaining the clock stopped.
- Letter from applicant to EFSA received on 30 May 2017 providing additional information.

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- Email from EFSA to applicant dated 31 May 2017 acknowledging the reception of the information received on 30 May 2017 and maintaining the clock stopped.
- Letter from applicant to EFSA received on 15 June 2017 providing additional information.
- Email from EFSA to applicant dated 15 June 2017 acknowledging the reception of the information received on 15 June 2017 and maintaining the clock stopped.
- Letter from applicant to EFSA received on 19 June 2017 providing additional information.
- Email from EFSA to applicant dated 20 June 2017 re-starting the clock as of 19 June 2017.
- Letter from EFSA to applicant dated 6 July 2017 requesting additional information and stopping the clock.
- Letter from EFSA to applicant dated 19 July 2017 requesting additional information and maintaining the clock stopped.
- Letter from EFSA to applicant dated 2 August 2017 requesting additional information and maintaining the clock stopped.
- Letter from applicant to EFSA received on 22 August 2017 providing additional information (request dated 6 July 2017).
- Letter from applicant to EFSA received on 22 August 2017 providing additional information (request dated 2 August 2017).
- Letter from EFSA to applicant dated 22 September 2017 requesting additional information and maintaining the clock stopped.
- Letter from applicant to EFSA received on 6 October 2017 providing additional information.
- Letter from EFSA to applicant dated 17 October 2017 Clarification teleconference
- Letter from EFSA to applicant dated 7 November 2017 requesting additional information and maintaining the clock stopped.
- Letter from EFSA to applicant dated 4 December 2017 requesting additional information and maintaining the clock stopped.
- Letter from applicant to EFSA received on 20 December 2017 providing additional information (requested dated 22 September 2017).
- Letter from applicant to EFSA received on 20 December 2017 providing additional information (requested dated 7 November 2017).
- Email from EFSA to applicant dated 21 December 2017 acknowledging the reception of the information received on 20 December 2017 (requested dated 22 September 2017) and maintaining the clock stopped.
- Email from EFSA to applicant dated 21 December 2017 acknowledging the reception of the information received on 20 December 2017 (requested dated 7 November 2017) and maintaining the clock stopped.
- Letter from EFSA to applicant dated 17 January 2018 requesting additional information and maintaining the clock stopped.
- Letter from applicant to EFSA received on 5 February 2018 providing additional information.
- Letter from applicant to EFSA received on 28 February 2018 providing additional information.
- Email from EFSA to applicant dated 1 March 2018 re-starting the clock as of 28 February 2018.
- Letter from EFSA to applicant dated 28 March 2018 requesting additional information and stopping the clock.
- Letter from applicant to EFSA received on 5 April 2018 providing additional information spontaneously.
- Letter from applicant to EFSA received on 6 April 2018 providing additional information.
- Email from EFSA to applicant dated 9 April 2018 re-starting the clock as of 6 April 2018.
- Letter from applicant to EFSA received on 31 May 2018 providing additional information spontaneously.

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Abbreviations

AOSA	Association of Official Seed Analysts
APTT	activated partial thromboplastin time
CRW	corn rootworms
DDE	daily dietary exposure
dsRNA	double-stranded RNA
ELISA	enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	environmental risk assessment
FOB	functional observation battery
GLP	good laboratory practice
GMO	genetically modified organism
HGT	horizontal gene transfer
HR	homologous recombination
IgE	immunoglobulin E
JSA	junction sequence analysis
LTP	lipid transfer protein
MCH	mean corpuscular haemoglobin
MS	mass spectrometry
NEP	Newly expressed protein
NGS	next generation sequencing
NcRNA	Non-coding RNA
ORF	open reading frames
PCR	polymerase chain reaction
PMEM	post-market environmental monitoring
PT	prothrombin time
RNAi	RNA interference
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
siRNA	Small interfering RNA

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