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Assessment of genetically modified maize MON 87403 for food and feed uses, import and processing, under Regulation (EC) No 1829/2003 (application EFSA-GMO-BE-2015-125)

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Abstract

Maize MON 87403 was developed to increase ear biomass at early reproductive phase through the expression of a modified *AtHB17* gene from *Arabidopsis thaliana*, encoding a plant transcription factor of the HD-Zip II family. The molecular characterisation data and bioinformatic 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 87403 and its conventional counterpart were identified. The compositional analysis of maize MON 87403 did not identify differences that require further assessment. The GMO Panel did not identify safety concerns regarding the toxicity and allergenicity of the AtHB17Δ113 protein, as expressed in maize MON 87403. The nutritional value of food and feed derived from maize MON 87403 is not expected to differ from that of food and feed derived from non-genetically modified (GM) maize varieties. Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, the GMO Panel concludes that maize MON 87403 is as safe and nutritious as the conventional counterpart and the non-GM maize reference varieties tested. In the case of accidental release of viable maize MON 87403 grains into the environment, maize MON 87403 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 87403. In conclusion, the GMO Panel considers that maize MON 87403, 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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Amendment: Reference to the publication Devos et al. 2018 was removed from the scientific output. This does not materially affect the content or outcome. The original version was removed from the EFSA Journal, but is available on request.

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Summary

Following the submission of the application EFSA-GMO-BE-2015-125 under Regulation (EC) No 1829/2003 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 87403 (unique identifier MON-874Ø3-1). The scope of the application EFSA-GMO-BE-2015-125 is for import, processing, and food and feed uses of maize MON 87403 within the European Union (EU), but excludes cultivation in the EU.

The GMO Panel evaluated maize MON 87403 with reference to the scope of the application EFSA-GMO-BE-2015-125, and appropriate principles described in its guidelines for the risk assessment of GM plants. The evaluation addresses the following components of the risk assessment: the molecular characterisation of the inserted DNA and analysis of the expression of the corresponding protein; the comparative analyses of compositional, agronomic and phenotypic characteristics; the safety of the newly expressed protein and the whole food and feed with respect to potential toxicity, allergenicity and nutritional characteristics; the environmental risk assessment; and the post-market environmental monitoring plan.

The molecular characterisation data establish that maize MON 87403 contains a single insert consisting of one copy of the AtHB17Δ113 protein expression cassette. Bioinformatic analyses of the sequence encoding the newly expressed protein and other open reading frames within the insert, or spanning the junctions between the insert and genomic DNA, did not indicate significant similarities to toxins and allergens. 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 AtHB17Δ113 protein was considered adequate. The protein characterisation data comparing the structural and biochemical properties of plant- and microbial-derived AtHB17Δ113 proteins indicate that this protein is equivalent and that the microbial produced protein can be used in the safety studies.

No statistically significant differences in the agronomic, phenotypic and physiological characteristics between maize MON 87403 and its conventional counterpart were identified, except for R1 and R6 ear biomass, ear partitioning, total kernel weight and total kernel number for which the combined-site analysis of 13 field trial sites showed that these were higher for maize MON 87403 compared to the conventional counterpart. As there was only partial overlap among the sites used for the agronomic, phenotypic and compositional characterisation of maize MON 87403 and those used for its physiological characterisation, the GMO Panel verified whether the intended trait, increased R1 ear biomass, was observed in the sites used for the compositional analysis. Based on the provided data, four out of seven sites from which samples were taken for the compositional analysis, phenotypic manifestation of the intended trait was realised. For these sites, the ear biomass (at the R1 or R6 stage) was higher. However, only for one site, the increase in ear biomass was statistically significant at the R1 and R6 stages, which raised the question on whether compositional data obtained from the field trials would allow a thorough risk assessment. The GMO Panel acknowledges that the change due to the intended trait is known to be of limited amplitude, and that the AtHB17Δ113 protein is expressed in maize MON 87403, which suggests that the manifestation of the trait may depend on environmental conditions in the field trials. The GMO Panel concludes that the agronomic, phenotypic and compositional analysis did not identify issues requiring further assessment regarding food and feed safety and its environmental impact.

The GMO Panel did not identify safety concerns regarding the toxicity and allergenicity of the AtHB17Δ113 protein, as expressed in maize MON 87403. The nutritional value of food and feed derived from maize MON 87403 is not expected to differ from that of food and feed derived from non-GM maize varieties. Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, the GMO Panel concludes that maize MON 87403 is as safe and nutritious as the conventional counterpart and the non-GM maize reference varieties tested.

Considering the introduced trait, the outcome of the comparative analysis, and the routes and levels of exposure, the GMO Panel concludes that maize MON 87403 would not raise safety concerns in the case of accidental release of viable GM maize grains into the environment. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of maize MON 87403.

The literature searches did not identify relevant publications that were not already submitted as part of the application. In the context of post-market environmental monitoring, the applicant should improve the literature searches according to the GMO Panel recommendations.

In delivering its scientific opinion, the GMO Panel took into account the application EFSA-GMO-BE-2015-125, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications. The GMO Panel concludes that maize MON 87403, 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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1. Introduction

The scope of the application EFSA-GMO-BE-2015-125 is for food and feed uses, import and processing of maize MON 87403 and does not include cultivation in the European Union (EU).

Maize MON 87403 was developed to increase ear biomass at early reproductive phase (Rice et al., 2014), which can provide an opportunity for increased grain yield under field conditions (Leibman et al., 2014).[1] This is achieved by the expression of the full-length *AtHB17* transcribed region from *Arabidopsis thaliana*, which in maize undergoes specific splicing, resulting in a protein truncated for the first 113 N-terminal amino acids (AtHB17Δ113).

Members of the plant HD-Zip transcription factor family have a leucine zipper motif (LZ) immediately C-terminal to the homeodomain (HD). These transcription factors typically bind as dimers to DNA, with the HD domain ensuring specific DNA binding, and the LZ domain functioning as a dimerisation motif (Ariel et al., 2007).

The AtHB17 protein from *A. thaliana* belongs to class II HD-Zip transcription factors which are characterised by containing a putative transcription repression domain N-terminal of the HD domain.[2]

When expressed in maize, the transcript is spliced to produce a truncated protein that lacks the first 113 N-terminal amino acids, leading to the loss of the repression domain (Rice et al., 2014). The AtHB17Δ113 protein still contains its HD and LZ domains and may interact with other HD-Zip II family transcription factors preventing their repressor activity (Rice et al., 2014). As maize HD-Zip II proteins are predominantly expressed in the ear tissue, the AtHB17Δ113 transcription factor may modulate HD-Zip II regulated pathways in the ear.[3]

1.1. Background

On 26 June 2015, the European Food Safety Authority (EFSA) received from the Competent Authority of Belgium the application EFSA-GMO-BE-2015-125 for authorisation of maize MON 87403 (Unique Identifier MON 874Ø3-1), submitted by Monsanto Europe within the framework of Regulation (EC) No 1829/2003 on GM food and feed.[4]

After receiving the application EFSA-GMO-BE-2015-125, 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.[5] 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. On 11 September 2015, EFSA received additional information requested under completeness check on 7 August 2015. On 2 October 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 the 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 following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion.[6] Member States had three months after the date of receipt of the valid application (until 2 January 2016) to make their opinion known.

The EFSA Panel on Genetically Modified Organisms (GMO Panel) requested additional information from the applicant on 23 October 2015 (EURL-JRC), 4 January 2016, 11 February 2016, 26 April 2016, 23 May 2016, 20 July 2016, 29 September 2016, 17 November 2016, 5 December 2016, 22 February 2017, 11 May 2017, 12 July 2017, 2 August 2017, 18 August 2017 and 7 November 2017. The applicant provided the requested information on 18 March 2016, 7 March 2016, 3 May 2016, 30 August 2016, 22 August 2016, 22 September 2016, 3 January 2017, 27 March 2017 (including the partial dataset received on 18 January 2017), 6 February 2017, 24 April 2017, 10 July 2017, 3 October 2017, 13 October 2017, 20 November 2017 and 12 February 2018, respectively.

[1] Dossier: Part II – Section 1.2.2.1.
[2] Dossier: Part II – Section 1.2.2.1 and 1.4.1.
[3] Dossier: Part II – Section 1.2.2.1; Additional information: 30/8/2016 and 3/1/2017.
[4] 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.
[5] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2015-00430.
[6] 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.

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 bioinformatic and statistical analyses, respectively.

In giving its scientific opinion on maize MON 87403 to the European Commission, the Member States and the applicant, and in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003, EFSA has endeavoured to respect a time limit of 6 months from the acknowledgement of the valid application. As additional information was requested by the GMO Panel, the time limit of 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 87403 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003.

Where applicable, any conditions or restrictions which should be imposed on the placing on the market and/or specific conditions or restrictions for use and handling, including post-market monitoring requirements based on the outcome of the risk assessment and, in the case of GMOs or food 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 the application EFSA-GMO-BE-2015-125, 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 87403 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 also assessed the applicant's systematic literature searches in accordance with the guidelines on literature searching given in EFSA (2010, 2017).

The comments raised by the Member States are addressed in Annex G of EFSA's overall opinion[5] and were taken into consideration during the scientific risk assessment.

3. Assessment

3.1. Systematic literature review[7]

The GMO Panel assessed the systematic literature searches provided by the applicant on maize MON 87403 according to the guidelines given in EFSA (2010, 2017).

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[7] Dossier: Part II – Section 7.

A systematic literature 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-BE-2015-125, because of the limited number of relevant publications on maize MON 87403.[8]

Although the overall quality of the performed literature searches is acceptable, the GMO Panel considers that the searches on maize MON 87403 could be improved. The GMO Panel therefore recommends the applicant to: design broader and more sensitive searches in the context of maize MON 87403; 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); include controlled vocabulary (subject indexing) in the searches when available (in addition to text words); use truncation consistently; use NEAR/5 (or greater) instead of NEAR/3; adapt the search to the size of the retrieved publications (and thus not combine search sets when one of the search sets already yields only a small number of publications); report the number of publications retrieved for each single search set performed (or search lines); and 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[9]

3.2.1. Transformation process and vector constructs[10]

Maize MON 87403 was developed by *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*)-mediated transformation.[11] Immature maize (*Z. mays* L., line LH244) embryos were cocultured with the *A. tumefaciens* strain ABI containing the binary transformation plasmid PV-ZMAP5714.[12]

The plasmid PV-ZMAP5714 used for the transformation contains a single *AtHB17* expression cassette between the right and left borders of the T-DNA. This expression cassette contains the following genetic elements: a chimeric promoter obtained by combining the duplicated enhancer region from the *Cauliflower mosaic virus* 35S RNA promoter with the promoter of the actin 1 (*act1*) gene from *Oryza sativa*; the 5′ UTR leader sequence from the chlorophyll a/b binding protein from *Triticum aestivum*; the intron and flanking UTR sequence of the *act1* gene; the coding sequence of the *AtHB17* gene from *A. thaliana* encoding a member of the class II homeodomain-leucin zipper gene family; and the 3′ UTR sequence from a heat shock protein (Hsp17) from *T. aestivum*.

The PV-ZMAP5714 vector also contains the *cp4 epsps* expression cassette, which consists of the promoter, leader, intron and flanking UTR sequence of *act1* gene from *O. sativa*, the targeting sequence of the *shkG* gene from *A. thaliana*, the coding sequence of the *aroA* gene from *Agrobacterium* sp. strain CP4, and the 3′ UTR sequence of the *nos* gene from *A. tumefaciens* pTi. The CP4 *epsps* expression cassette was also integrated during transformation (Huang et al., 2004) at a different locus. This allowed for glyphosate to be used for transformant screening and subsequent segregation for the selection of plants only containing the *AtHB17* expression cassette.

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[13]

Molecular characterisation of maize MON 87403 was performed by next generation sequencing (NGS) and junction sequence analysis (JSA), polymerase chain reaction (PCR), DNA sequence analysis and Southern 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 both in terms of coverage and sensitivity.

[8] Additional information: 13/10/2017 and 12/2/2018.
[9] Dossier: Part II – Section 1.2.
[10] Dossier: Part II – Sections 1.2.1.1, 1.2.1.2 and 1.2.1.3.
[11] Dossier: Part II – Section 1.2.1.1.
[12] Dossier: Part II – Section 1.2.1.2.
[13] Dossier: Part II – Sections 1.2.2.1, 1.2.2.2 and 1.2.2.5.
[14] Dossier: Part II – Section 1.2.2.2.

Whole genome analysis by NGS/JSA indicated that the maize MON 87403 contains a single insert, which consists of a single copy of T-DNA in the same configuration as in the PV-ZMAP5714 transformation vector.[15] Junction sequence pairs detection confirmed the insert and copy number of the T-DNA. Whole genome analysis by NGS/JSA also indicated the absence of vector backbone sequences.

The nucleotide sequence of the entire insert of maize MON 87403 together with 1,345 bp of the 5′ and 1,267 bp of the 3′ flanking regions were determined. The insert of 3,132 bp is identical to the T-DNA of PV-ZMAP5714, except for the deletion of 333 bp of the right border region and 211 bp of the left border region.[16]

A comparison with the pre-insertion locus indicated that 149 bp were deleted from the maize genomic DNA.[17] The possible interruption of known endogenous maize genes by the insertion in maize MON 87403 was evaluated by bioinformatic analyses of the pre-insertion site and of the genomic sequences flanking the insert. The results of these analyses did not indicate the interruption of any known endogenous gene in maize MON 87403.

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

Updated bioinformatic analyses of the amino acid sequence of the newly expressed AtHB17Δ113 protein revealed no significant similarities to known toxins and allergens. In addition, updated bioinformatic 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.[14]

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 for event MON 87403. The likelihood and potential consequences of plant-to-microorganisms gene transfer are described in Section 3.5.1.2.

3.2.3. Protein characterisation and equivalence[18]

Maize MON 87403 expresses one new protein, AtHB17Δ113. Given the technical restraints in producing large enough quantities from plants for safety testing, AtHB17Δ113 was recombinantly produced in *Escherichia coli.* Prior to safety studies, a set of biochemical methods was employed to demonstrate the equivalence between maize and microbe-derived proteins. Purified proteins from these sources were characterised and compared in terms of their physicochemical, structural and functional properties.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis showed that both plant- and microbe-derived AtHB17Δ113 proteins migrated close to the expected molecular weight of approximately 22 kDa and were immunoreactive to a specific polyclonal antibody, as shown by western blot analysis. Amino acid sequence analysis by mass spectrometry suggested that both proteins matched the deduced sequence as defined by the *AtHB17Δ113* gene. These data also showed that a fraction of the protein had its N-terminal methionine truncated. Additional AtHB17Δ113 fractions were shown to be acetylated in methionine 1 or asparagine 2. In contrast, the N-terminus of the microbe-produced protein was intact. Such modifications are common in eukaryotic proteins (e.g. Polevoda and Sherman, 2000). Sequence analysis by mass spectrometry also indicated that the plant-derived AtHB17Δ113 protein was not glycosylated. Due to the low yield and purity of the plant-derived AtHB17Δ113 protein, functional equivalence was indirectly demonstrated by assessing the activity of only the *E. coli*-produced AtHB17Δ113 protein and evaluating the results in the context of available information on the activity of other *Arabidopsis* HD-Zip II proteins. Using a qualitative enzyme-linked immunosorbent assay (ELISA)-based protein-DNA endpoint assay, it was shown that*, in vitro*, the *E. coli*-produced AtHB17Δ113 protein can bind to DNA with a specific sequence as expected.[19]

Therefore, the GMO Panel accepts the use of the AtHB17Δ113 protein produced in bacteria for the safety studies.

[15] Dossier: Part II – Section 1.2.2.2.a; Additional information: 3/5/2016.
[16] Dossier: Part II – Section 1.2.2.2.b.
[17] Dossier: Part II – Section 1.2.2.2.c.
[18] Dossier: Part II – Section 1.4.1.1; Study: MSL0025829.
[19] Study: MSL0026645; Additional information: 30/8/2016.

3.2.4. Information on the expression of the insert[20]

Protein levels of AtHB17Δ113 were analysed by ELISA in material harvested from replicated field trials across five locations in the USA during the 2012 growing season. Samples analysed included leaves (V3–V4, V6–V9, V10–V12 and ~ VT), root (V3–V4, V6–V9, V10–V12, ~ VT, R5 and R6), whole plant (V3–V4, V6–V9, V10–V12 and ~ VT), forage (R5), stover (~ R6), pollen (R1), grain (R6) and silk tissue (~ R1).[20] The mean values, standard deviations and ranges of protein expression levels in grains (n = 20) and forage (n = 20) of the AtHB17Δ113 protein are summarised in Table 1.

Table 1: Means, standard deviations and ranges of protein levels in grains and forage (μg/g dry weight) from maize MON 87403

Event	Grain (R6)	Forage (R5)
AtHB17Δ113	< LOD	0.0018[(a)] ± 0.00064[(b)]
	N/A–N/A	0.0011 – 0.0035[(c)]

LOD: limit of detection.
(a): Mean.
(b): Standard deviation.
(c): Range.

3.2.5. Inheritance and stability of inserted DNA[21]

Genetic stability of the MON 87403 insert was assessed by NGS/JSA and Southern blot[7] analyses for five generations. The obtained data from coverage mapping and junction sequence classes (JSCs) were sufficient to conclude that all the plants tested retained the single copy of the insert and flanking regions, which were stably inherited in subsequent generations.[21] Stability was also observed by segregation PCR analysis of the construct that expresses the AtHB17Δ113 protein in plants from three generations of maize MON 87403. The results supported the presence of a single insertion, segregating in a Mendelian fashion.

3.2.6. Conclusion on molecular characterisation

The molecular characterisation data establish that maize MON 87403 contains a single insert consisting of one copy of the AtHB17Δ113 protein expression cassette. Bioinformatic analyses of the sequence encoding the newly expressed protein and other ORFs within the insert, or spanning the junctions between the insert and genomic DNA, did not indicate significant similarities to toxins and allergens. 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 AtHB17Δ113 protein was considered adequate. The protein characterisation data comparing the structural and biochemical properties of plant- and microbe-derived AtHB17Δ113 proteins indicated that these proteins are equivalent and the microbe-produced protein can be used in the 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-BE-2015-125 presents data on agronomic and phenotypic characteristics, as well as forage and grain composition, of maize MON 87403 derived from field trials performed at nine sites in the USA in 2012.[24] In addition, the application contains data on characteristics of pollen and seed from maize MON 87403, and data on additional phenotypic endpoints illustrating the effect of the genetic modification, hereafter referred to as physiological characteristics. The latter were derived from complementary field trials and a greenhouse study (Table 2).

[20] Dossier: Part II – Section 1.2.2.3.
[21] Dossier: Part II – Section 1.2.2.4.
[22] Dossier: Part II – Section 1.3; Additional information: 7/3/2016, 6/7/2016, 27/3/2017 (including the partial dataset received on 18/1/2017) and 10/7/2017.
[23] Dossier: Part II – Sections 1.3.1 and 1.3.2.
[24] A total of nine sites were used for the agronomic/phenotypic and compositional studies, of which seven were used for both the agronomic/phenotypic and compositional analysis. The NCBD site was used only for the agronomic/phenotypic assessment, while the IALL site was included only for the compositional analysis.

Table 2: Overview of comparative assessment studies with maize MON 87403 provided in the application EFSA-GMO-BE-2015-125

Study focus	Study details	Comparators	Commercial non-GM maize reference varieties
Agronomic and phenotypic analysis	Field trials, 2012, USA (8 locations)	MPA640B	19
	Seed germination test	MPA640B	4
	Pollen germination test	MPA640B	4
Compositional analysis	Field trials, 2012, USA (8 locations)	MPA640B	17
Physiological analysis	Field trials, 2012, USA (13 locations)	MPA640B	None
	Greenhouse study	MPA640B	None

Non-GM: non-genetically modified.

Field trials with maize MON 87403 were conducted in major maize-growing areas of the USA, representing regions of diverse agronomic practices and environmental conditions. For the agronomic and phenotypic and compositional studies, maize MON 87403, the conventional counterpart (MPA640B) and four non-GM maize reference varieties, all treated (sprayed) with required maintenance pesticides (including conventional herbicides), were grown in a randomised complete block design with four replicates at each site.[25] In total (across sites), up to 19 non-GM commercial maize reference varieties were included in the field trials (Table 2).[26] The comparator used in the field trials is a non-GM maize line (MPA640B [= LH244×LH287]) with a genetic background similar to that of maize MON 87403 (as documented by the pedigree[27]), and was therefore considered to be the appropriate conventional counterpart.

For the physiological field trials, at each site, maize MON 87403 and the conventional counterpart, all treated (sprayed) with required maintenance pesticides (including conventional herbicides), were grown in a randomised complete block design with four replicates, except at one site where the field trial consisted of three replicates.[28] A subset of these 13 sites, respectively, 8 and 7 sites, was also used for the agronomic and phenotypic and compositional analyses.[29]

3.3.2. Statistical analysis of field trial data

The statistical analysis of the agronomic and phenotypic and compositional data from the 2012 field trials followed the recommendations of the GMO Panel (EFSA GMO Panel, 2010b, 2011a) and complied with Regulation (EU) No 503/2013. This included 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).[30]

[25] For the agronomic/phenotypic analysis, the field sites were located in: Jackson, Arkansas (ARNE); Jefferson, Iowa (IARL); Warren, Illinois (ILMN); Boone, Indiana (INSH); Pawnee, Kansas (KSLA); Perquimans, North Carolina (NCBD); Polk, Nebraska (NESH); and Lehigh, Pennsylvania (PAGR). For the compositional analysis, the field sites were located in: Jackson, Arkansas (ARNE); Story, Iowa (IALL); Jefferson, Iowa (IARL); Warren, Illinois (ILMN); Boone, Indiana (INSH); Pawnee, Kansas (KSLA); Polk, Nebraska (NESH); and Lehigh, Pennsylvania (PAGR).

[26] For the agronomic/phenotypic analysis, a total of 19 different non-GM maize reference varieties were evaluated, i.e. Burrus 645, Gateway 4148, Gateway 6158, H-9180, Legacy L7671, Lewis 6442, Lewis 7007, LG2540, LG2620, Midland Phillips 799, Mycogen 2M746, NC+ 4443, NC+ 5220, Phillips 713, Phillips 717, Pioneer 32T16, Stewart S588, Stewart S602, Stine 9724. For the compositional analysis, a total of 17 different non-GM maize reference varieties were evaluated, i.e. Burrus 645, Gateway 4148, Gateway 6158, H-9180, Lewis 6442, Lewis 7007, LG2540, LG2620, Midland Phillips 799, Mycogen 2M746, NC+ 4443, NC+ 5220, Phillips 713, Phillips 717, Stewart S588, Stewart S602 and Stine 9724.

[27] Dossier: Part II – Section 1.2.2.2 (see Figure 8).

[28] The field sites were located in: Jackson, Arkansas (ARNE); Greene, Iowa (IABG); Jefferson, Iowa (IARL); Vermilion, Illinois (ILCX); Warren, Illinois (ILMN); Boone, Indiana (INSH); Pawnee, Kansas (KSLA); Perquimans, North Carolina (NCBD); Butler, Nebraska (NEDC); Polk, Nebraska (NESH); York, Nebraska (NEYO); Lehigh, Pennsylvania (PAGR), and Berks, Pennsylvania (PAHM).

[29] For the agronomic/phenotypic analysis, the field sites were: ARNE, IARL, ILMN, INSH, KSLA, NCBD, NESH and PAGR. For the compositional analysis, the field sites were: ARNE, IARL, ILMN, INSH, KSLA, NESH and PAGR.

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

3.3.3. Agronomic and phenotypic analysis[31]

3.3.3.1. Agronomic and phenotypic characteristics tested under field conditions

The agronomic and phenotypic parameters evaluated in the 2012 field trials were: early stand count, days to 50% pollen shed, days to 50% silking, dropped ear count, ear height, plant height, final stand count, root lodged plants, stalk lodged plants, stay green, grain moisture, test weight, yield, visually observable responses to naturally occurring diseases (disease incidence), arthropod damage and abiotic stress responses.[32]

Of the 16 endpoints evaluated, 13[33] could be analysed with the combination of difference and equivalence testing described, while the three remaining endpoints[34] were not subjected to statistical analysis because they did not meet the assumptions for analysis of variance.

For none of the 13 endpoints analysed, statistically significant differences were identified between maize MON 87403 and the conventional counterpart.

Additionally, no altered stress responses of maize MON 87403 were observed compared with its conventional counterpart with regard to visually observable responses to naturally occurring diseases, arthropod damage and abiotic stressors.

3.3.3.2. Agronomic and phenotypic characteristics tested under controlled conditions

Seed characteristics

The applicant tested the germination rate of seeds harvested from maize MON 87403 (selfed F_2 seeds), the conventional counterpart and four non-GM maize varieties.[35] Germination was tested in growth chambers under controlled conditions at seven different temperature regimes. The endpoints analysed were the numbers of normal germinated seeds, abnormal germinated seeds, hard seeds, dead seeds and firm swollen seeds. No statistically significant differences in the germination rate of seeds were observed between maize MON 87403 and its conventional counterpart.

Although the applicant referred to seed dormancy when discussing the generated data on maize MON 87403 seed characteristics, no data on induced seed dormancy were supplied. Therefore, the GMO Panel considers that only the conclusions on seed germination of maize MON 87403 are substantiated by the provided data. Given that the genetic modification of maize MON 87403 is not designed to target specific seed characteristics, that maize is not a persistent and invasive crop, and that the scope of the application EFSA-GMO-BE-2015-125 excludes cultivation, the GMO Panel does not consider data on seed dormancy needed for the risk assessment of maize MON 87403.

Pollen characteristics

The applicant reported data on pollen characteristics of maize MON 87403. The endpoints analysed were pollen diameter and viability via the Alexander stain method. No significant difference between maize MON 87403 and its conventional counterpart was observed for pollen diameter and general pollen morphology. Although Alexander's stain is intended to provide an indication of pollen viability, it does not directly measure pollen viability (Dafni, 1992). Therefore, the data on pollen viability supplied by the applicant in support of the comparative assessment of maize MON 87403 are not considered suitable by the GMO Panel. Given that the genetic modification of maize MON 87403 is not designed to target specific pollen characteristics, that maize is not a persistent and invasive crop and that the scope of the application EFSA-GMO-BE-2015-125 excludes cultivation, the GMO Panel considers that data provided on pollen are not required for the risk assessment of maize MON 87403.

3.3.3.3. Physiological characteristics tested under field conditions

Physiological characteristics of maize MON 87403 and the conventional counterpart were assessed and compared under field conditions.[36] Information on R1 and R6 ear biomass, R1 and R6 stover

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[31] Dossier: Part II – Section 1.3.5; Additional information: 7/3/2016, 6/7/2016, 27/3/2017 (including the partial dataset received on 18/1/2017) and 10/7/2017.

[32] For the endpoint early stand count, data for only three replications at the PAGR site were initially reported in the application, but complemented with data collected from another row (see Additional information: 7/3/2016).

[33] The endpoints were: early stand count, days to 50% pollen shed, days to 50% silking, dropped ear count, ear height, plant height, final stand count, root lodged plants, stalk lodged plants, stay green, grain moisture, test weight and yield.

[34] The endpoints were: visually observable responses to naturally occurring diseases (disease incidence), arthropod damage and abiotic stress responses.

[35] Test materials were collected from three sites of the 2012 field trials: Story, Iowa (IALL); Warren, Illinois (ILMN); and Lehigh, Pennsylvania (PAGR).

[36] Additional information: 7/3/2016, 6/7/2016, 27/3/2017 (including the partial dataset received on 18/1/2017) and 10/7/2017.

biomass, R1 and R6 total plant biomass, ear partitioning, ear biomass, total kernel weight, total kernel number, single kernel weight, harvest index and dry matter production was gathered from field trials conducted at 13 sites in 2012 (Table 2).

The R1 and R6 ear biomass, ear partitioning, total kernel weight and total kernel number were statistically significantly higher in maize MON 87403 than in the conventional counterpart in the combined-site analysis. For the other endpoints, no statistically significant difference was observed between maize MON 87403 and its conventional counterpart in the combined-site analysis.

Site-by-site analyses were supplied by the applicant for the endpoints R1 and R6 ear biomass and total kernel weight. These analyses indicated that the R1 ear biomass was statistically significantly higher for maize MON 87403 than for the conventional counterpart at the NESH and NEYO sites, of which the NESH site was also used for the agronomic and phenotypic and compositional analyses, and higher at seven other sites, of which three sites (ILMN, KLSA and INSH) were also used for the agronomic and phenotypic and compositional analyses. The R6 ear biomass was shown to be statistically significantly higher for maize MON 87403 than for the conventional counterpart at the ILMN site, which was also used for the agronomic and phenotypic and compositional analyses, and higher at eight other sites, of which three sites (KSLA, IARL and PAGR) were also used for the agronomic and phenotypic and compositional analyses. The total kernel weight was statistically significantly higher for maize MON 87403 than for the conventional counterpart at the ILMN site, which was used for the agronomic and phenotypic and compositional analyses, and higher at eight other sites, of which three sites (IARL, KLSA and PAGR) were also used for the agronomic and phenotypic and compositional analyses.

3.3.3.4. Physiological characteristics tested under controlled conditions

The applicant assessed physiological characteristics of maize MON 87403 and the conventional counterpart in pots under optimal growing conditions in a greenhouse.[37] The endpoints analysed were husk biomass, cob biomass, ear biomass, stover biomass, total biomass, ear partitioning, ear diameter and ear length at each growth stage, and number of ovule rows and total ovule number at the late R1 stage. It was shown that: husk biomass, cob biomass and number of ovule rows were statistically significantly higher for maize MON 87403 at the late R1 stage than its conventional counterpart; cob biomass was statistically significantly higher for maize MON 87403 at the late R1 stage; ear biomass, ear partitioning and ear diameter were statistically significantly higher for maize MON 87403 at the early and late R1 stages; net ear biomass was statistically significantly higher for maize MON 87403 at the V16 and late R1 stages; and cob biomass, stover biomass and total biomass were statistically significantly lower for maize MON 87403 at the V16 stage. No statistically significant differences in husk biomass at the V16, VT and early R1 stages, cob biomass at the VT and early R1 stages, ear biomass at the V16 and VT stages, stover and total biomass at the VT, early R1 and late R1 stages, ear partitioning at the V16 and VT stages, ear diameter at the VT and V16 stages, ear length at any growth stage, and total ovule number at the late R1 stage were observed between maize MON 87403 and its conventional counterpart.

3.3.4. Compositional analysis[38]

Maize forage and grains harvested from the field trials in the USA in 2012 were analysed for 78 different constituents (nine in forage[39] and 69 in grains[40]), including the key constituents

[37] Additional information: 7/3/2016, 6/7/2016 and 27/3/2017 (including the partial dataset received on 18/1/2017).

[38] Dossier: Part II – Sections 1.3.3 and 1.3.4.

[39] The endpoints were: protein, fat, ash, moisture, carbohydrates by calculation, ADF, NDF, calcium and phosphorus.

[40] The endpoints were: protein, fat, ash, moisture, carbohydrates by calculation, acid detergent fibre (ADF), neutral detergent fibre (NDF), total dietary fibre (TDF), alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), myristoleic acid (C14:1), pentadecanoic acid (C15:0), pentadecenoic acid (C15:1), palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), heptadecenoic acid (C17:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), γ-linolenic acid (C18:3), nonadecanoic acid (C19:0), arachidic acid (C20:0), eicosenoic acid (C20:1), eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3), arachidonic acid (C20:4), behenic acid (C22:0), β-carotene, thiamin, riboflavin, pyridoxine, α-tocopherol, niacin, folic acid, calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, zinc, phytic acid, raffinose, furfural, ferulic acid, and *p*-coumaric acid.

recommended by the OECD (2002). Sixteen grain constituents having more than 50% of the observations below the limit of quantification were excluded from the statistical analysis.[41]

The test of difference and the test of equivalence could be applied to the remaining 62 constituents, with the following results. The test of difference identified statistically significant differences from the conventional counterpart for three constituents (one in forage (calcium) and two in grains (riboflavin and moisture)). The test of equivalence between maize MON 87403 and the non-GM maize reference varieties indicated that all three constituents fell under equivalence category I.

The GMO Panel assessed all significant differences between maize MON 87403 and its conventional counterpart taking into account their potential impact on the plant's metabolism and the natural variability observed for the set of non-GM maize reference varieties. No endpoints showing significant differences between maize MON 87403 and its conventional counterpart and falling under category III/IV were identified.

3.3.5. Conclusion on comparative analysis

No statistically significant differences in the agronomic, phenotypic and physiological characteristics between maize MON 87403 and its conventional counterpart were identified, except for R1 and R6 ear biomass, ear partitioning, total kernel weight and total kernel number for which the combined-site analysis of 13 field trial sites showed that these were higher for maize MON 87403 compared to the conventional counterpart. As there was only partial overlap among the sites used for the agronomic, phenotypic and compositional characterisation of maize MON 87403 and those used for its physiological characterisation, the GMO Panel verified whether the intended trait, increased R1 ear biomass, was observed in the sites used for the compositional analysis. Based on the provided data, four out of seven sites from which samples were taken for the compositional analysis, phenotypic manifestation of the intended trait was realised. For these sites, the ear biomass (at the R1 or R6 stage) was higher. However, only for one site the increase in ear biomass was statistically significant at the R1 and R6 stages, which raised the question on whether compositional data obtained from the field trials would allow a thorough risk assessment. The GMO Panel acknowledges that the change due to the intended trait is known to be of limited amplitude, and that the AtHB17Δ113 protein is expressed in maize MON 87403, which suggests that the manifestation of the trait may depend on environmental conditions in the field trials. The GMO Panel concludes that the agronomic, phenotypic and compositional analysis did not identify issues requiring further assessment regarding food and feed safety and its environmental impact.

3.4. Food and feed safety assessment[42]

3.4.1. Effects of processing[43]

Based on the outcome of the comparative assessment (Section 3.3.5), processing of maize MON 87403 into food and feed products is not expected to result in products different from those of commercial non-GM maize varieties.

3.4.2. Influence of temperature and pH on the newly expressed protein[44]

The effect of heating on the functional activity of the bacterially produced AtHB17Δ113 protein was evaluated. The AtHB17Δ113 protein was partially labile when heated at 75°C or above for 15 or 30 min. In addition, SDS–PAGE analysis showed that heating up to 75°C had little or no effect on the integrity of the AtHB17Δ113 protein. An appearance of lower molecular weight bands were observed when the protein was heated at 95°C.

The effect of pH on the AtHB17Δ113 protein stability was assessed by SDS–PAGE analysis at three pH conditions: pH 7.4 (the protein's storage buffer), pH 1.2 and pH 7.5. The data showed that the bacterially produced AtHB17Δ113 proteins remain intact at these pH solutions.

[41] These were: sodium, furfural, caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), myristoleic acid (C14:1), pentadecanoic acid (C15:0), pentadecenoic acid (C15:1), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), heptadecenoic acid (C17:1), γ-linolenic acid (C18:3), eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3) and arachidonic acid (C20:4).

[42] Dossier: Part II – Sections 1.4, 1.5, 1.6 and 2; Additional information: 17/11/2017, 20/9/2016, 7/3/2016, 19/12/2016 and 10/4/2017.

[43] Dossier: Part II – Section 1.3.6.

[44] Dossier: Part II – Sections 1.4.1.3 and 1.4.1.4.

3.4.3. Toxicology[45]

3.4.3.1. Testing of the newly expressed protein[46]

Molecular and biochemical characterisation of the newly expressed protein

Maize MON 87403 expresses one new protein, AtHB17Δ113. This protein has been characterised in its structure and function (see Section 3.2).

Bioinformatics

Bioinformatic analysis of its amino acid sequence revealed no relevant similarities to known toxic proteins (see Section 3.2).

Similarity to proteins in food and feed

An *in silico* study was carried out to assess the sequence similarity with proteins from other plant species,[47] using the AtHB17Δ113 amino acid sequence as a query. The results indicated that a sequence identity of AtHB17Δ113 to related proteins ranged from ~ 67% to 83%, with the highest identity to proteins from *Brassica* species (*Brassica rapa* and *Brassica olearacea*). When the AtHB17Δ113 protein sequence region composed of the HD and LZ functional domains was used as a query, 61–77% identity was observed with proteins from a number of plant species consumed as food and feed (e.g. grape, tomato, potato, sweet orange, soybean, rice, maize).

In vitro *degradation studies*[48]

The resistance of the bacterial AtHB17Δ113 protein to degradation by pepsin was investigated in solutions at pH ~ 1.2. The integrity of the test protein in probes taken at various time points was analysed by SDS–PAGE followed by protein staining or by western blot. The full-length AtHB17Δ113 protein was degraded by pepsin within 30 s. At this time point, a protein fragment of 4–5 kDa remained visible, but it disappeared at the 2 min incubation time point. By western blot analysis, none of these fragments were immunologically reactive to an AtHB17Δ113 antibody.

In addition, the applicant performed a standalone degradation study of the bacterial AtHB17Δ113 protein in a so-called simulated intestinal fluid (SIF) according to a method previously described (USP, 1995). The GMO Panel notes that the resistance to degradation by standalone SIF is currently not specifically required by either EFSA GMO Panel (2011a) or Codex Alimentarius (2009). Due to the intrinsic limitations of such standalone SIF degradation study for the food and feed safety of the newly expressed protein, it was not considered in the overall safety assessment.

Acute oral toxicity testing

A bacterial AtHB17Δ113 protein was administered by gavage at the dose of 1,335 mg/kg body weight (bw) to male and female Crl:CD1(ICR) mice. No adverse effects related to the AtHB17Δ113 protein were observed.

Based on the above-mentioned information on the AtHB17Δ113 protein and considering its high identity to proteins present in several plants used as food and feed, the GMO Panel concludes that no additional toxicological studies are needed to conclude on the safety of this protein.

3.4.3.2. Testing of new constituents other than the newly expressed protein[49]

Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, no new constituents other than newly expressed protein has been identified in seed and forage from maize MON 87403 (Section 3.2.3). Therefore, no further food and feed safety assessment of components other than the newly expressed protein is required.

[45] Dossier: Part II – Section 1.4; Additional information: 20/9/2016, 19/12/2016, 7/3/2016, 10/4/2017 and 17/11/2017.
[46] Dossier: Part II – Section 1.4.1; Additional information: 17/11/2017.
[47] Dossier: Part II – Section 1.4.1.1; Additional information: 17/11/2017.
[48] Dossier: Part II – Section 1.5.1.3.
[49] Dossier: Part II – Section 1.4.2.

3.4.3.3. Information on altered levels of food and feed constituents[50]

Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, none of the differences identified between maize MON 87403 and its conventional counterpart in seed and forage composition (Section 3.3.4) require further assessment.

3.4.3.4. Testing of the whole genetically modified food or feed[51]

Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, no substantial modifications in the composition of maize MON 87403 and no indication of possible unintended effects relevant for food and feed safety were identified. Therefore, animal studies on the food and feed derived from maize MON 87403 are not necessary (EFSA GMO Panel, 2011a). In accordance to Regulation (EU) No 503/2013, the applicant provided a 90-day oral repeated dose toxicity study on whole food and feed from maize MON 87403 in rats. Animal feeding studies in broiler and channel catfish fed diets containing maize MON 87403 material were also provided in compliance with Regulation (EU) No 503/2013. All these studies were evaluated by the GMO Panel.

90-day feeding study in rats

Pair-housed Crl:CD(SD) rats (16/sex per group, 7-week-old) were allocated to two groups using a randomised complete block design with eight replications. Groups were fed a test or control diet containing approximately 33% (weight/weight) of maize MON 87403 (test item) or from the conventional counterpart MPA640B (control material), respectively. The study provided was adapted from OECD TG 408 (OECD, 1998), and compliant with the principles of Good Laboratory Practice.

Event-specific PCR analysis carried out on grains prior to processing of these grains into meal confirmed the molecular identity of maize MON 87403. The event MON 87403 was not detected in the conventional counterpart. Both test items and control materials were analysed for proximates, amino acids, minerals, antinutrients and the presence of pesticides. Balanced diets were prepared according to the specifications for PMI Certified Rodent LabDiet #5002 (grounded maize included at a rate of approximately 33% (weight/weight)). The diets are considered stable for the duration of the treatment, based on expiration standards declared by the diet manufacturer. Diet preparation procedures and regular evaluations of the mixing methods by surrogate analytes guaranteed their homogeneity and the proper concentration of the test or control substances.

Feed and water were provided *ad libitum*. Animals were checked twice daily for mortality and clinical signs. Detailed clinical examinations were conducted on all animals pretreatment and then weekly during the dosing period, and on the day of the scheduled necropsy. Individual body weights were recorded pretreatment 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, functional observations data and motor activity were recorded on all animals pre-treatment and at the end of the study. Clinical pathology (i.e. haematology, clinical chemistry and coagulation, urine analyses) and necropsy examination with organs weighing were conducted at the end of the treatment period on all animals. The animals were not fed 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 histopathological assessment of all tissues, histopathology was reviewed by a peer review pathologist.

Mean, median, standard deviation, minimum and maximum 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 prespecified effect sizes[52] for eight endpoints[53] with a 5% level of significance. For almost all selected endpoints, the power estimates was at least 80%.

The statistical analysis compared rats consuming the test diet with those consuming the control diet. The cage or the individual animal was considered the experimental unit according to the corresponding estimate of cage effect.

[50] Dossier: Part II – Section 1.4.3.
[51] Dossier: Part II – Section 1.4.4; Additional information: 20/9/2016, 19/12/2016, 7/3/2016 and 10/4/2017.
[52] Defined on the basis of six previous studies: WIL-50283 (Kirkpatrick, 2005), WIL-50296 (Kirkpatrick, 2007), WIL-50297 (Kirkpatrick, 2007), WIL-50333 (WIL-WIL-50370 (Kirkpatrick, 2010)); Additional information: 20/9/2016.
[53] Eight endpoints were selected: absolute lymphocytes, alkaline phosphatase, body weight, cholesterol, creatinine, urea nitrogen, kidney weight and liver weight.

The in-life and terminal body weights, cumulative body weight changes, organ weights, feed consumption/efficiency and clinical pathology and functional observations when appropriate, parameters were checked for homogeneity and normality,[54] analysed with analysis of variance (ANOVA) and tested using a t-tests. Finally, outcome proportions of incidence of functional observations were analysed with the Fisher's exact test. In response to a request from EFSA,[55] 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).[56] The goodness of fit was evaluated by visual examination of residual plots and histograms.[57] Based on this evaluation, the models were considered appropriate.

One female rat fed the test diet died while being anaesthetised for blood collection on the day of the scheduled necropsy; the GMO Panel considers this death accidental and not related to treatment. No test diet related clinical signs and ophthalmoscopic findings were observed.

No statistically significant differences in mean body weight and cumulative body weight changes were observed in the groups administered test diet compared to the control group.

Higher feed intake was noted in the first and in the last week of the treatment period in males given the test-diet compared to controls; the GMO Panel notes that this increase in feed intake is not associated with differences in the body weight and cumulative body weight changes, and thus not toxicologically relevant.

A statistically significantly shorter mean time to first step was observed in the open field observations for the test-substance treated female group compared to the control group. This was the only change among the 11 parameters examined in the open field observations and among the 45 parameters examined in the functional observational battery and therefore, not considered to be test-substance related.

In the locomotor activity examinations, statistically significantly higher mean cumulative and ambulatory counts were noted for the test-substance-treated female group compared to the control group. These changes, without any correlation with other relevant functional observational battery and locomotor activity examinations, were not considered to be toxicologically relevant.

No statistically significant differences were noted between test-diet fed rats and control regarding haematology, coagulation, clinical chemistry, urinalysis and organ weights.

The macroscopic examination performed at necropsy on all animals, revealed no gross pathological findings related to the administration of the test material in the diet. The microscopic examinations of selected organs and tissues did not identify relevant differences in the incidence and severity of the histopathological findings related to the administration of the test diet.

The GMO Panel notes that the applicant only tested one dose level. However, the dose tested was close to the highest possible without inducing nutritional imbalance according to current knowledge and in accordance with the limit test (OECD, 1998). This is considered not to compromise the study (EFSA, 2014).

The GMO Panel concludes that no adverse effects were observed after feeding a diet including 33% of maize MON 87403 to rats for 90 days.

42-day broiler study[58]

A total of 800 (400/sex) one-day old chicken broilers (Cobb x Cobb 500) were randomly allocated to eight dietary groups with 100 chicks per treatment (10 pens/treatment, half for each sex, 10 birds/pen) and fed balanced diets[59] containing up to 62%[60] of grain from maize MON 87403 (test diet), the conventional counterpart MPA640B (control diet) or one of the six non-GM maize reference varieties Gateway 4148, Stewart S602, Stine 9724, Legacy L7671, NC+433 and Jacobsen seed JS443 (reference diets). Diets (as crumble pellets or pellet) and water were offered *ad libitum*.

No statistically significant differences between the groups fed test and control diets were observed in the majority of growth performance parameters: mortality (about 1.5%), final body weight, weight gain, feed to gain ratio, and yield of prechilled organs and postchilled carcass and cuttable parts (absolute and relative weights). A difference in adjusted feed to gain ratio was observed between the

[54] Normality and heterogeneity assumptions were checked by visual examination of residual plots and histogram. No extreme violations of the assumptions were observed.
[55] Additional information: 20/9/2016 and 19/12/2016.
[56] Additional information: 19/12/2016.
[57] Additional information: 10/4/2017.
[58] Additional information: 7/3/2016.
[59] Starter (0–21 days), grower/finisher (22–42 days) diets.
[60] ~ 58% in starter diets; ~ 62% in grower/finisher diets.

test group and one of the six reference groups. Because no other statistically significant differences were observed between the test group and the remaining reference groups, the one observed is not considered relevant.

The GMO Panel concludes that administration of diets containing up to 62% of maize MON 87403 to broilers did not cause adverse effects in this study, and that the measured performance endpoints were similar between groups fed balanced diets containing GM and non-GM maize (conventional counterpart and non-GM maize reference varieties).

8-week channel fish study[58]

A total of 600 channel catfish (sex undetermined) were randomly allocated to six dietary groups with 100 catfishes per treatment (five aquaria/treatment, 20 fishes/aquaria) and fed balanced diets formulated as sinking pellets containing approximately 32% of ground maize grain from maize MON 87403 (test diet), the conventional counterpart MPA640B (control diet) or one of the four non-GM maize reference varieties Gateway 4148, Stewart S602, Stine 9724 and Legacy L7671 (reference diets).

No treatment-related mortality and no abnormal behaviours were observed among fish 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 test, control and reference diets.

The GMO Panel concludes that administration of balanced diets containing up to 32% of maize MON 87403 to channel catfish did not cause adverse effects in this study and that the measured performance endpoints were similar between groups fed balanced diets containing GM and non-GM maize (conventional counterpart and non-GM maize reference varieties).

3.4.4. Allergenicity[61]

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 protein[62]

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, 2010c, 2011a).

The *AtHB17* gene originates from *A. thaliana*, an organism that is not considered to be a common allergenic source.

Updated bioinformatic analyses of the amino acid sequences of the AtHB17Δ113 protein, using the criterion of 35% identity in a sliding window of 80 amino acids, revealed no significant similarities to known allergens.

The study on resistance to degradation of the AtHB17Δ113 protein by pepsin has been described in Section 3.4.3.1.

There is no information available on the structure or function of the newly expressed AtHB17Δ113 protein that would suggest an adjuvant effect of the protein in maize MON 87403 resulting in or enhancing an eventual specific immunoglobulin E (IgE) response to a bystander protein.

In the context of the present application, the GMO Panel considers that there are no indications that the newly expressed AtHB17Δ113 protein in maize MON 87403 may be allergenic.

3.4.4.2. Assessment of allergenicity of the whole GM plant or crop[63]

The GMO Panel regularly reviews the available publications on food allergy to maize. However, to date, maize has not been considered to be a common allergenic food (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 87403 were compared to those in the conventional counterpart MPA640B. No statistically significant differences between them were observed.

Based on the outcome of the studies considered in the comparative analysis and the molecular characterisation, and the assessment of the newly expressed protein (Sections 3.2, 3.3 and 3.4), the

[61] Dossier: Part II – Section 1.5; Additional information: 7/3/2016.
[62] Dossier: Part II – Sections 1.5.1 and 1.5.3.
[63] Dossier: Part II – Section 1.5.2; Additional information: 7/3/2016.

GMO Panel found no reasons of concern regarding the allergenicity of food and feed derived from maize MON 87403 with respect to that derived from its conventional counterpart.

3.4.5. Dietary exposure assessment to endogenous and new constituents[64]

In line with Regulation (EU) No 503/2013, dietary exposure to the AtHB17Δ113 protein was considered for the risk assessment of maize MON 87403.

3.4.5.1. Human dietary exposure[65]

Only acute dietary exposure estimates to the AtHB17Δ113 protein 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 limit of detection (LOD) (0.00025 μg/g fresh weight) was used as the assumed maximum amount of the AtHB17Δ113 protein present in maize MON 87403 grains (including sweet corn); this assumption most probably overestimates the real concentration of the AtHB17Δ113 protein in maize grains. Consumption figures were retrieved by the applicant from the available summary statistics present in the EFSA Comprehensive European Food Consumption Database.[66] 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). A conservative scenario with 100% replacement of conventional maize by the GM maize was considered. Food commodities derived from maize for which no proteins are expected to occur (e.g. maize oil, maize syrup, bourbon whiskey among others) were excluded.

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),[67] and multiplying these values by the reported LOD for the AtHB17Δ113 protein. Processing factors or recipes that may decrease the amount of the AtHB17Δ113 protein found in the consumed commodities as compared to that in maize grains were not considered. Among the young population, the highest acute exposure would be achieved in toddlers following the consumption of sweet corn (0.002 μg/kg bw per day), while for adults the consumption of popcorn would lead to the highest acute exposure estimate (0.001 μg/kg bw per day). The use of the highest acute consumption for only one food commodity could slightly underestimate the dietary exposure to the AtHB17Δ113 protein in certain population groups.

The GMO Panel estimated chronic dietary exposure to the AtHB17Δ113 protein, which was not provided by the applicant. Individual consumption data on food commodities from dietary surveys with at least two days consumption and covering a total of 19 European countries[68] were retrieved from the EFSA Comprehensive European Food Consumption Database. As for the acute exposure estimations provided by the applicant, the LOD of the AtHB17Δ113 protein was used as the maximum assumed protein concentration in maize grains. Different recipes and processing factors were considered to estimate the amount of maize in the consumed commodities before assigning the AtHB17Δ113 protein levels to the relevant commodities.[69] No losses in the AtHB17Δ113 protein during processing were considered. The highest chronic dietary exposure was estimated for toddlers with a highest mean chronic dietary exposure of 0.00035 μg/kg bw per day, and a 95th percentile exposure of 0.0012 μg/kg bw per day across dietary surveys. Corresponding highest estimates in adults were 0.000067 μg/kg bw per day and 0.00034 μg/kg bw per day for the mean and 95th percentile exposure, respectively. Overall, in those dietary surveys with the highest chronic exposure the main contributor to the dietary exposure were corn flakes.

[64] Dossier: Part II – Section 2.

[65] Dossier: Part II – Section 2.4.

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

[67] 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

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

[69] 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 0.00008 μg of AtHB17Δ113/g of corn bread.

3.4.5.2. Animal dietary exposure[70]

Daily dietary exposure (DDE) to the AtHB17Δ113 protein in maize MON 87403 was provided by the applicant across different livestock animal species (broiler, finishing pig and lactating dairy cow) based on estimates, as 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 meal in animal diets. A conservative scenario with 100% replacement of the conventional maize (grain, gluten feed and gluten meal) by the GM maize was considered. All the grain samples analysed in maize MON 87403 for the presence of the AtHB17Δ113 protein were below the LOD (0.00028 μg/g dry weight). For the purpose of estimating DDE, the LOD was used as the assumed mean amount of protein in grain, and as a reference to estimate protein levels in gluten feed and gluten meal, calculated to be, respectively, 2.6- and 7.1-fold higher than in grain, based on the assumption that no protein is lost during the processing and protein content of gluten feed and gluten meal relative to maize grain (OECD, 2002). Estimated DDE to AtHB17Δ113, based on the total consumption of GM maize grain, gluten feed and gluten meal was 0.033 μg/kg bw in broiler, 0.016 μg/kg bw in finishing pig and 0.027 μg/kg bw in lactating dairy cow.

The GMO Panel estimated DDE to the AtHB17Δ113 protein 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 that was 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 AtHB17Δ113 protein in forage, derived from the field trials performed in 2012 (see Table 1), were used as occurrence data. Estimated DDEs to the AtHB17Δ113 protein, based on the consumption of GM maize forage/silage was 0.034 μg/kg bw in beef, 0.041 μg/kg bw in dairy cow, 0.022 μg/kg bw in lamb and 0.008 μg/kg bw in breeding swine.

3.4.6. Nutritional assessment of GM food and feed[71]

The intended trait of maize MON 87403 is increased R1 ear biomass, with no intention to alter the nutritional parameters. Comparison of the grains and forage composition of maize MON 87403 with the conventional counterpart and the non-GM maize reference varieties did not identify differences that would require a nutritional assessment as regards food and feed (see Section 3.3.4).

Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, the GMO Panel concludes that the nutritional impact of maize MON 87403 derived food and feed is similar to that expected from its conventional counterpart and non-GM maize reference varieties.

3.4.7. Post-market monitoring of GM food and feed[72]

No biologically relevant compositional, agronomic and phenotypic changes were identified in maize MON 87403 when compared with its conventional counterpart. The GMO Panel therefore considers maize MON 87403 to be as safe as the conventional counterpart and that post-market monitoring (EFSA GMO Panel, 2011a) of the food and feed derived from maize MON 87403 is not necessary.

3.4.8. Conclusions on the food and feed safety assessment

The GMO Panel did not identify safety concerns regarding the toxicity and allergenicity of the AtHB17Δ113 protein, as expressed in maize MON 87403. The nutritional value of food and feed derived from maize MON 87403 is not expected to differ from that of food and feed derived from non-GM maize varieties. Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, the GMO Panel concludes that maize MON 87403 is as safe and nutritious as the conventional counterpart and the non-GM maize reference varieties tested.

3.5. Environmental risk assessment and monitoring plan[73]

Considering the scope of the application EFSA-GMO-BE-2015-125, which excludes cultivation, the ERA of maize MON 87403 mainly takes into account: (1) the exposure of microorganisms to

[70] Dossier: Part II – Section 2.3.
[71] Dossier: Part II – Section 1.6.
[72] Dossier: Part II – Section 4.
[73] Dossier: Part II – Sections 5 and 6.

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 87403 grains during transportation and processing (EFSA GMO Panel, 2010a).

3.5.1. Environmental risk assessment[74]

3.5.1.1. Persistence and invasiveness of the GM plant[75]

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, the absence of a dormancy phase and susceptibility to plant pathogens, herbivores and cold climate conditions (OECD, 2002). 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 trait of event MON 87403 will provide a selective advantage to maize plants. Therefore, the presence of the intended trait will not affect the persistence and invasiveness of the GM plant.

In conclusion, the GMO Panel considers it very unlikely that maize MON 87403 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 87403 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.

Plant-to-microorganism gene transfer[76]

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 domesticated 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 homologous recombination. 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 87403 does not express recombinant genes encoded by bacterial DNA. Bioinformatic analyses of the recombinant DNA of maize MON 87403 revealed a single DNA sequence of sufficient length and identity to bacterial DNA to facilitate homologous recombination, i.e. the right border sequence with T-DNA of *A. tumefaciens*. No pairs of DNA sequences with sufficient identity to bacterial DNA, which would facilitate HGT by double homologous recombination, were identified.

In summary, there is no indication for an increased likelihood of horizontal transfer of DNA from maize MON 87403 to bacteria. Given the nature of the recombinant DNA, the GMO Panel identified no safety concern linked to an unlikely but theoretically possible HGT.

[74] Dossier: Part II – Section 5.
[75] Dossier: Part II – Section 5.3.1.
[76] Dossier: Part II – Section 5.3.2.

Plant-to-plant gene transfer[75]

The potential for occasional feral maize MON 87403 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 for the reasons given in Section 3.5.1.1.

3.5.1.3. Interactions of the GM plant with target organisms[77]

Taking the scope of the application EFSA-GMO-BE-2015-125 (no cultivation) and thus the absence of target organisms into account, potential interactions of occasional feral maize MON 87403 plants arising from grain import spills with the target organisms are not considered a relevant issue by the GMO Panel.

3.5.1.4. Interactions of the GM plant with non-target organisms[78]

Given that environmental exposure of non-target organisms to spilled GM grains or occasional feral GM maize plants arising from spilled maize MON 87403 grains is limited and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions of the maize MON 87403 with non-target organisms are not considered by the GMO Panel to raise any environmental safety concern.

3.5.1.5. Interactions with the abiotic environment and biogeochemical cycles[79]

Given that environmental exposure to spilled grains or occasional feral maize MON 87403 plants arising from grain import spills is limited and because most proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions with the abiotic environment and biogeochemical cycles are not considered by the GMO Panel to raise any environmental safety concern.

3.5.2. Post-market environmental monitoring[80]

The objectives of a PMEM plan, according to Annex VII of Directive 2001/18/EC, are to: (1) 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 rationale of the PMEM plan provided by the applicant (EFSA GMO Panel, 2011b).

As the ERA did not identify potential adverse environmental effects from the maize MON 87403, no case-specific monitoring is required.

[77] Dossier: Part II – Section 5.3.3.
[78] Dossier: Part II – Section 5.3.4.
[79] Dossier: Part II – Section 5.3.6.
[80] Dossier: Part II – Section 6.

The PMEM plan proposed by the applicant for maize MON 87403 includes: (1) the description of an approach involving operators (federations involved in maize 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 newly established by EuropaBio for the collection of the 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 post-market environmental monitoring 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 87403. 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 87403 would differ from conventional maize varieties in its ability to persist under European environmental conditions. Considering the scope of the application EFSA-GMO-BE-2015-125, interactions of occasional feral maize MON 87403 plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from maize MON 87403 to bacteria does not indicate a safety concern. Therefore, considering the introduced trait, the outcome of the comparative analysis, the routes and levels of exposure, the GMO Panel concludes that maize MON 87403 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 87403.

4. Conclusions

The GMO Panel was asked to carry out a scientific assessment of maize MON 87403 for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003.

The molecular characterisation data establish that maize MON 87403 contains a single insert consisting of one copy of the AtHB17Δ113 protein expression cassette. Bioinformatic analyses of the sequence encoding the newly expressed protein and other ORFs within the insert, or spanning the junctions between the insert and genomic DNA, did not indicate significant similarities to toxins and allergens. 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 AtHB17Δ113 protein was considered adequate. The protein characterisation data comparing the structural and biochemical properties of plant- and microbe-derived AtHB17Δ113 proteins indicated that these proteins are equivalent and the microbe-produced protein can be used in the safety studies.

No statistically significant differences in the agronomic, phenotypic and physiological characteristics between maize MON 87403 and its conventional counterpart were identified, except for R1 and R6 ear biomass, ear partitioning, total kernel weight and total kernel number for which the combined-site analysis of 13 field trial sites showed that these were higher for maize MON 87403 compared to the conventional counterpart. As there was only partial overlap among the sites used for the agronomic, phenotypic and compositional characterisation of maize MON 87403 and those used for its physiological characterisation, the GMO Panel verified whether the intended trait, increased R1 ear biomass, was observed in the sites used for the compositional analysis. Based on the provided data, four out of seven sites from which samples were taken for the compositional analysis, phenotypic manifestation of the intended trait was realised. For these sites, the ear biomass (at the R1 or R6 stage) was higher. However, only for one site the increase in ear biomass was statistically significant at the R1 and R6 stages, which raised the question on whether compositional data obtained from the field trials would allow a thorough risk assessment. The GMO Panel acknowledges that the change due to the intended trait is known to be of limited amplitude, and that the AtHB17Δ113 protein is expressed in maize MON 87403, which suggests that the manifestation of the trait may depend on environmental conditions in the field trials. The GMO Panel concludes that the agronomic, phenotypic and compositional analysis did not identify issues requiring further assessment regarding food and feed safety and its environmental impact.

The GMO Panel did not identify safety concerns regarding the toxicity and allergenicity of the AtHB17Δ113 protein, as expressed in maize MON 87403. The nutritional value of food and derived from maize MON 87403 is not expected to differ from that of food and feed derived from non-GM maize varieties. Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, the GMO Panel concludes that maize MON 87403 is as safe and nutritious as the conventional counterpart and the non-GM maize reference varieties tested.

The GMO Panel concludes that there is a very low likelihood of environmental effects resulting from the accidental release of viable grains from maize MON 87403 into the environment. The PMEM plan and reporting intervals are in line with the intended uses of maize MON 87403.

Based on the relevant publication retrieved through systematic literature searches, the GMO Panel did not identify any safety issues pertaining to the intended uses of maize MON 87403. In the context of PMEM, the applicant should improve the literature searches according to the GMO Panel recommendations.

In conclusion, the GMO Panel considers that maize MON 87403, 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

1) Letter from the Competent Authority of Belgium received on 26 June 2015 concerning a request for placing on the market of genetically modified maize MON 87403 submitted by Monsanto Europe S.A./N.V. in accordance with Regulation (EC) No 1829/2003 (application reference EFSA-GMO-BE-2015-125).
2) Acknowledgement letter dated 13 July 2015 from EFSA to the Competent Authority of Belgium.
3) Letter from EFSA to applicant dated 7 August 2015 requesting additional information under completeness check.
4) Letter from applicant to EFSA received on 11 September 2015 providing a timeline for submission of responses.
5) Letter from EFSA to applicant dated **2 October 2015** delivering the 'Statement of Validity' of the application EFSA-GMO-BE-2015-125 for placing on the market of genetically modified MON 87403 submitted by Monsanto Europe S.A./N.V. in accordance with Regulation (EC) No 1829/2003.
6) Letter from EURL-JRC to EFSA dated 16 October 2015 requesting EFSA to stop the clock.
7) Letter from EFSA to applicant dated 23 October 2015 requesting additional information (EURL-JRC) and stopping the clock.
8) Letter from applicant to EFSA received on 19 November 2015 providing additional information.
9) Letter from EFSA to applicant dated 4 January 2016 requesting additional information and remaining the clock stopped.
10) Letter from EFSA to applicant dated 11 February 2016 requesting additional information and remaining the clock stopped.
11) Letter from applicant to EFSA received on 7 March 2016 providing additional information.
12) Email from EURL-JRC to EFSA dated 1 April 2016 requesting EFSA to re-start the clock.
13) Letter from EFSA to applicant dated 26 April 2016 requesting additional information and remaining the clock stopped.
14) Letter from applicant to EFSA received on 3 May 2016 providing additional information.
15) Letter from EFSA to applicant dated 23 May 2016 requesting additional information and remaining the clock stopped.
16) Letter from applicant to EFSA received on 6 July 2016 providing additional information.
17) Letter from EFSA to applicant dated 20 July 2016 requesting additional information and remaining the clock stopped.
18) Letter from applicant to EFSA received on 20 July 2016 extending the timeline for submission of responses.
19) Letter from applicant to EFSA received on 22 August 2016 providing additional information.
20) Letter from applicant to EFSA received on 30 August 2016 providing additional information.
21) Letter from applicant to EFSA received on 22 September 2016 providing additional information.

22) Email from EFSA to applicant dated 23 September 2016 re-starting the clock on 22 September 2016.
23) Letter from EFSA to applicant dated 29 September 2016 requesting additional information and stopping the clock.
24) Letter from EFSA to applicant dated 17 November 2016 requesting additional information and remaining the clock stopped.
25) Letter from applicant to EFSA received on 28 November 2017 extending the timeline for submission of responses.
26) Letter from EFSA to applicant dated 5 December 2016 requesting additional information and remaining the clock stopped.
27) Letter from applicant to EFSA received on 3 January 2017 providing additional information.
28) Letter from applicant to EFSA received on 18 January 2017 providing additional information.
29) Letter from applicant to EFSA received on 6 February 2017 providing additional information.
30) Email from EFSA to applicant dated 7 February 2017 re-starting the clock on 6 February 2017.
31) Letter from EFSA to applicant, dated 15 February 2017 requesting clarifications concerning additional information provided on 18 January 2017.
32) Letter from EFSA to applicant dated 22 February 2017 requesting additional information and stopping the clock.
33) Letter from applicant to EFSA, dated 24 February 2017 providing clarifications concerning additional information provided on 18 January 2017.
34) Letter from applicant to EFSA received on 27 March 2017 providing additional information concerning clarifications requested by EFSA on 15 February 2017.
35) Letter from applicant to EFSA received on 24 April 2017 providing additional information.
36) Email from EFSA to applicant dated 28 April 2017 re-starting the clock on 24 April 2017.
37) Letter from EFSA to applicant dated 11 May 2017 requesting additional information and stopping the clock.
38) Letter from applicant to EFSA received on 10 July 2017 providing additional information.
39) Email from EFSA to applicant dated 10 July 2017 re-starting the clock on 10 July 2017.
40) Letter from EFSA to applicant dated 12 July 2017 requesting additional information and stopping the clock.
41) Letter from EFSA to applicant dated 2 August 2017 requesting additional information and remaining the clock stopped.
42) Letter from EFSA to applicant dated 18 August 2017 requesting additional information and remaining the clock stopped.
43) Letter from applicant to EFSA received on 3 October 2017 providing additional information.
44) Letter from applicant to EFSA received on 13 October 2017 providing additional information.
45) Letter from EFSA to applicant dated 7 November 2017 requesting additional information and remaining the clock stopped.
46) Letter from applicant to EFSA received on 20 November 2017 providing additional information.
47) Letter from applicant to EFSA received on 19 December 2017 extending the timeline for submission of responses.
48) Letter from applicant to EFSA received on 12 February 2018 providing additional information.
49) Email from EFSA to applicant dated 13 February 2018 re-starting the clock on 12 February 2018.

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Abbreviations

ADF	acid detergent fibre
ANOVA	analysis of variance
bp	base pair
bw	body weight
DDE	daily dietary exposure
ELISA	enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	environmental risk assessment
FAO	Food and Agricultural Organization of the United Nations
GM	genetically modified
GMO	genetically modified organism
GMO Panel	EFSA Panel on Genetically Modified Organisms
HD	homeodomain
HGT	horizontal gene transfer
HSP	heat shock protein
IgE	immunoglobulin E
JSC	junction sequence class
JSA	junction sequence analysis
LOD	limit of detection
LTP	lipid transfer protein
LZ	leucine zipper motif
NDF	neutral detergent fibre
NGS	next generation sequencing
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PCR	polymerase chain reaction
PMEM	post-market environmental monitoring
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis

SIF	simulated intestinal fluid
TDF	total dietary fibre
T-DNA	transfer-deoxyribonucleic acid
UTR	untranslated region