Assessment of genetically modified maize MZIR098 for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-DE-2017-142)

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SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 28 May 2020

doi: 10.2903/j.efsa.2020.6171

Assessment of genetically modified maize MZIR098 for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-DE-2017-142)

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Abstract

Maize MZIR098 was developed to confer tolerance to glufosinate-ammonium-containing herbicides and resistance to certain coleopteran pests. The molecular characterisation data and bioinformatic analyses do not identify issues requiring food/feed safety assessment. None of the identified differences in the agronomic/phenotypic and compositional characteristics tested between maize MZIR098 and its conventional counterpart needs further assessment, except for neutral detergent fibre (NDF) in grains, which does not raise nutritional and safety concerns. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the eCry3.1Ab, mCry3A and PAT proteins as expressed in maize MZIR098, and finds no evidence that the genetic modification would change the overall allergenicity of maize MZIR098. In the context of this application, the consumption of food and feed from maize MZIR098 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that maize MZIR098 is as safe as the conventional counterpart and non-GM maize reference varieties tested, and no post-market monitoring of food/feed is considered necessary. In the case of accidental release of viable maize MZIR098 grains into the environment, maize MZIR098 would not raise environmental safety concerns. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of maize MZIR098. In conclusion, the GMO Panel considers that maize MZIR098, as described in this application, is as safe as its conventional counterpart and the non-GM maize reference varieties tested with respect to potential effects on human and animal health and the environment.

Keywords: GMO, maize (*Zea mays*), MZIR098, Regulation (EC) No 1829/2003, eCry3.1Ab, mCry3A, PAT

Requestor: Competent Authority of Germany

Question number: EFSA-Q-2017-00398

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Acknowledgements: The Panel wishes to thank the members of the Working Groups on Molecular Characterisation, Food and Feed Safety Assessment and Working Group On Comparative Analysis and Environmental Risk Assessment for the preparatory work on this scientific output and EFSA staff members Silvia Federici, Irene Muñoz Guajardo, Sylvie Mestdagh, Oberkofler Lorenz and Irina Vlas for the support provided to this scientific output.

Suggested citation: EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Bresson J-L, Dalmay T, Dewhurst IC, Epstein MM, Firbank LG, Guerche P, Hejatko J, Moreno FJ, Mullins E, Nogué F, Rostoks N, Sánchez Serrano JJ, Savoini G, Veromann E, Veronesi F, Álvarez F, Ardizzone M, De Sanctis G, Devos Y, Dumont AF, Gennaro A, Gómez Ruiz JÁ, Lanzoni A, Neri FM, Papadopoulou N, Paraskevopoulos K and Raffaello T, 2020. Scientific Opinion on the assessment of genetically modified maize MZIR098 for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-DE-2017-142). EFSA Journal 2020;18(6):6171, 28 pp. https://doi.org/10.2903/j.efsa.2020.6171

ISSN: 1831-4732

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

Summary

In this scientific opinion, the scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (hereafter referred to as the 'GMO Panel') reports on the outcome of its risk assessment of maize MZIR098 according to the scope as defined in the application EFSA-GMO-DE-2017-142. The GMO Panel conducted the assessment of maize MZIR098 in line with the principles described in Regulation (EU) No 503/2013 and its applicable guidelines for the risk assessment of genetically modified (GM) plants.

The molecular characterisation data establish that maize MZIR098 contains a single insert consisting of one copy of the eCry3.1Ab, mCry3A and PAT expression cassettes. Updated bioinformatic analyses of the sequences encoding the newly expressed proteins and open reading frames (ORFs) present within the insert or spanning the junctions between the insert and genomic DNA, does not raise concerns that need additional food/feed safety considerations. The stability of the inserted DNA and of the introduced traits was confirmed over several generations. The methodology used to quantify the levels of the eCry3.1Ab, mCry3A and PAT proteins is considered adequate. The protein characterisation data comparing the structural, biochemical and functional properties of plant- and microbe-produced eCry3.1Ab, mCry3A and PAT proteins indicate that these proteins are equivalent, and the microbe-produced protein can be used in safety studies.

None of the identified differences in the agronomic/phenotypic and compositional characteristics tested between maize MZIR098 and its conventional counterpart needs further assessment, with the exception of neutral detergent fibre (NDF) in grains. The NDF in grains difference was further assessed for its safety and nutritional relevance and raises no concerns.

The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the eCry3.1Ab, mCry3A and PAT proteins as expressed in maize MZIR098, and finds no evidence that the genetic modification would change the overall allergenicity of maize MZIR098. In the context of this application, the consumption of food/feed from maize MZIR098 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that maize MZIR098 is as safe as the conventional counterpart and non-GM maize reference varieties tested, and no post-market monitoring of food/feed is considered necessary.

Considering the introduced traits, the outcome of the agronomic and phenotypic analysis and the routes and levels of exposure, maize MZIR098 would not raise safety concerns in the case of accidental release of viable GM maize grains into the environment. The post-market environmental monitoring (PMEM) plan and reporting intervals are in line with the intended uses of maize MZIR098.

Based on the relevant publications identified through the literature searches, the GMO Panel does not identify any safety issues pertaining to the uses of maize MZIR098. In the context of annual PMEM reports, the applicant could further fine-tune future literature searches according to the GMO Panel recommendations.

In delivering its scientific opinion, the GMO Panel took into account application EFSA-GMO-DE-2017-142, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications. The GMO Panel concludes that maize MZIR098, as described in this application, is as safe as its conventional counterpart and the non-GM maize reference varieties tested with respect to potential effects on human and animal health and the environment.

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1. Introduction

The scope of application EFSA-GMO-DE-2017-142 is for food and feed uses, import and processing of the genetically modified (GM) herbicide tolerant and insect resistant maize MZIR098 in the European Union (EU).

1.1. Background

On 2 May 2017, the European Food Safety Authority (EFSA) received from the Competent Authority of Germany application EFSA-GMO-DE-2017-142 for authorisation of maize MZIR098 (Unique Identifier SYN-ØØØ98-3), submitted by Syngenta Crop Protection N.V./S.A. (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003[1].

Following receipt of application EFSA-GMO-DE-2017-142, EFSA informed EU Member States and the European Commission, and made the application available to them. Simultaneously, EFSA published the summary of the application.[2]

EFSA checked the application for compliance with the relevant requirements of EFSA guidance documents, and, when needed, asked the applicant to supplement the initial application. On 11 August 2017, EFSA declared the application valid.

From validity date, EFSA and its scientific Panel on Genetically Modified Organisms (hereafter referred to as 'the GMO Panel') endeavoured to respect a time limit of six months to issue a scientific opinion on application EFSA-GMO-DE-2017-142. Such time limit was extended whenever EFSA and/or its GMO Panel requested supplementary information to the applicant. According to Regulation (EC) No 1829/2003, any supplementary information provided by the applicant during the risk assessment was made available to the EU Member States and European Commission (for further details, see the section 'Documentation', below).

In accordance with Regulation (EC) No 1829/2003, EFSA consulted the nominated risk assessment bodies of EU Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC[3]. The EU Member States had three months to make their opinion known on application EFSA-GMO-DE-2017-142 as of date of validity.

1.2. Terms of Reference as provided by the requestor

According to Articles 6 and 18 of Regulation (EC) No 1829/2003, EFSA and its GMO Panel were requested to carry out a scientific risk assessment of maize MZIR098 in the context of its scope as defined in application EFSA-GMO-DE-2017-142.

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).

The relevant information is made available in the EFSA Register of Questions including the information required under Annex II to the Cartagena Protocol; a labelling proposal; a Post-Market Environmental Monitoring (PMEM) plan as provided by the applicant; the method(s), validated by the Community reference laboratory, for detection, including sampling, identification of the transformation event in the food-feed and/or foods-feeds produced from it and the appropriate reference materials.[4]

2. Data and methodologies

2.1. Data

The GMO Panel based its scientific risk assessment of maize MZIR098 on the valid application EFSA-GMO-DE-2017-142, additional information provided by the applicant during the risk assessment, relevant scientific comments submitted by the Member States and relevant peer-reviewed scientific publications. In addition to this comprehensive information package, the GMO Panel also received unpublished studies submitted by the applicant in order to comply with the specific provisions of Regulation (EU) No 503/2013. A list of these additional unpublished studies is provided in Appendix A.

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.
[2] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2017-00398
[3] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 12.3.2001, p. 1–38.
[4] http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2017-00398

2.2. Methodologies

The GMO Panel conducted its assessment in line with the principles described in Regulation (EU) No 503/2013, its applicable guidelines (i.e. EFSA GMO Panel, 2010a,b, 2011a,b, 2015) and explanatory notes (i.e. EFSA, 2014, EFSA, 2017a,b) for the risk assessment of GM plants.

For the assessment of 90-day animal feeding studies, the GMO Panel took into account the criteria included in the EFSA Scientific Committee guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed (EFSA Scientific Committee, 2011) and the explanatory statement for its applicability (EFSA, 2014).

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

3. Assessment

3.1. Systematic literature review[5]

The GMO Panel assessed the applicant's literature searches on maize MZIR098, which included a scoping review, according to the guidelines given in EFSA (2010, 2017a).

A systematic review as referred to in the Commission Regulation (EU) No 503/2013 has not been provided in support to the risk assessment of application EFSA-GMO-DE-2017-142. Based on the outcome of the scoping review, the GMO Panel agrees that there is limited value of undertaking a systematic review for maize MZIR098 at present.

Although the overall quality of the performed literature searches is acceptable, the GMO Panel considers that future searches on maize MZIR098 could be fine-tuned further. The GMO Panel therefore recommends the applicant to 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).

Based on the relevant publications identified through the literature searches (Appendix B), the GMO Panel does not identify any safety issues pertaining to the intended uses of maize MZIR098.

3.2. Molecular characterisation[6]

3.2.1. Transformation process and vector constructs[7]

Maize MZIR098 was developed by *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*)-mediated transformation of maize (*Zea mays*) line NP2222 immature embryos with plasmid vector pSYN17629. A non-oncogenic helper plasmid pSB1 was also used in the transformation process.

The plasmid pSYN17629 used for the transformation contained three expression cassettes between the right and left borders of the T-DNA: *ecry3.1Ab*, *mcry3A* and *pat-08,* which was used as a selectable marker during the transformation process (Negrotto et al., 2000).

- The *ecry3.1Ab* expression cassette contains the following genetic elements: the enhancer sequence from the nopaline synthase gene (NOS enhancer) from *A. tumefaciens*; the promoter region from cestrum yellow leaf curling virus; the chimeric *ecry3.1Ab* gene, consisting of a fusion between a modified *cry3A* (*mcry3A*) and a synthetic, plant codon optimised *cry1Ab* from *Bacillus thuringiensis* subsp. *kurstaki* strain HD-1; and the terminator sequence from the nopaline synthase (*nos*) gene from *A. tumefaciens*.
- The *mcry3A* expression cassette contains the following genetic elements: the promoter region and first intron of the polyubiquitin gene from *Zea mays*; the synthetic *mcry3A* gene, a plant codon optimised *cry3A* gene from *B. thuringiensis* subsp. *tenebrionis* containing a consensus cathepsin G protease recognition site; and the terminator sequence from the *nos* gene from *A. tumefaciens*.

[5] Dossier: Part II – Section 7; Additional information: 30/11/2018.
[6] Dossier: Part II - Section 1.2.
[7] Dossier: Part II - Sections 1.2.1.1 and 1.2.1.2.

- The *pat* expression cassette contains the following genetic elements: the promoter region of the cauliflower mosaic virus; the plant codon optimised *pat* gene from *Streptomyces viridochromogenes* strain Tü494; and the terminator sequence from the *nos* gene from *A. tumefaciens*.

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

3.2.2. Transgene constructs in the GM plant[8]

Molecular characterisation of maize MZIR098 was performed by Southern analysis, polymerase chain reaction (PCR) combined with next-generation sequencing (NGS) and DNA sequence analysis, in order to determine insert copy number, size and organisation of the inserted sequences and to confirm the absence of plasmid backbone sequences. The approach used was acceptable both in terms of coverage and sensitivity.

Southern analyses indicated that maize MZIR098 contains a single insert, which consists of a single copy of the T-DNA in the same configuration as in the pSYN17629.[9] The insert and copy number were confirmed by multiple restriction enzyme/probe combinations covering the T-DNA region and flanking regions. PCR and NGS analyses confirmed the results obtained by the Southern analyses. The absence of vector backbone sequences was demonstrated by Southern analysis using two overlapping backbone-specific probes.

The nucleotide sequence of the entire insert of maize MZIR098 together with 1,000 bp of the 5′ and 3′ flanking regions were determined.[10] The insert of 8,467 bp is identical to the T-DNA of pSYN17629, except for the truncation of the border regions. A comparison of the flanking regions with the pre-insertion locus indicated that 24 bp of the parental genomic sequence had been deleted upon transformation.[11] The possible interruption of known endogenous maize genes by the insertion in event MZIR098 was evaluated by bioinformatic analyses of the pre-insertion locus and of the genomic sequences flanking the insert. The results of these analyses did not reveal the interruption of any known endogenous gene in the maize MZIR098.[12]

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 sequences of the newly expressed eCry3.1Ab, mCry3A and PAT proteins 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 or spanning the junctions between the insert and genomic DNA revealed that the expression of an ORF showing significant similarities to toxins or allergens is highly unlikely. A single ORF which exceeded the allergenicity assessment threshold of 35% identity using an 80 amino acid sliding window approach was further assessed.[13] This ORF is found within the transcriptional unit of the mCry3A coding sequence driven by the polyubiquitin promoter from *Z. mays*, it is in the same orientation and reading frame to the mCry3A ORF, and contains an in-frame translational start codon. For the assessment of this ORF, the GMO Panel followed a weight of evidence approach taking into account that, (i) the translational start codon is within an intron that is likely to be spliced out; (ii) experimental data obtained by two LC-MS/MS-based proteomic approaches did not show evidence for the presence of the putative protein derived from this ORF under the tested experimental conditions that allowed the detection of other MZIR098 maize proteins including mCry3A, eCry3.1Ab and PAT; and (iii) the sequence homology between the ORF and the known allergens is in a low-complexity amino acid region, that is known to produce random hits and that cannot be unequivocally linked to shared structures and/or allergenicity. Considering all this information, the GMO Panel is of the opinion that this ORF does not raise concerns that need additional food/feed safety considerations.

In order to assess the possibility for horizontal gene transfer by homologous recombination (HR), the applicant performed a sequence identity analysis of the regions of bacterial origin in maize MZIR098. The likelihood and potential consequences of plant-to-bacteria gene transfer are described in Section 3.5.1.2.

[8] Dossier: Part II - Section 1.2.2.2.
[9] Appendix 1.2.1.
[10] Appendix 1.2.2 and 1.2.3 (confidential information).
[11] Appendix 1.2.4 and 1.2.5 (confidential information).
[12] Additional information: 16/12/2019.
[13] Additional information: 20/04/18; 16/12/19; 26/2/20; 30/4/20.

3.2.3. Protein characterisation and equivalence[14]

Maize MZIR098 expresses three new proteins: eCry3.1Ab, mCry3A and PAT.

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

3.2.3.1. eCry3.1Ab characterisation and equivalence

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis showed that plant- and microbe-produced eCry3.1Ab proteins had the expected molecular weight of ~ 73.7 and 74.8 kDa, respectively, and were comparably immunoreactive to eCry3.1Ab protein-specific antibodies. The slight molecular weight difference was mainly due to the presence of the poly histidine tag (6xHis) at the N-terminus of the microbial-produced eCry3.1Ab. Glycosylation detection analysis demonstrated that none of the eCry3.1Ab proteins were glycosylated. Amino acid sequence analysis by mass spectrometry methods showed that both proteins matched the deduced sequence as defined by the *eCry3.1Ab* gene. These data also showed that the N-terminal methionine of the plant-produced eCry3.1Ab was truncated. Such modifications are common in eukaryotic proteins (e.g. Polevoda and Sherman, 2000). Functional equivalence was demonstrated by an insect-feeding bioassay which showed that the insecticidal activity of a plant extract containing an eCry3.1Ab:mCry3A mixture was similar to the activity of an microbial-produced eCry3.1Ab:mCry3A mixture of equal amounts to the plant-produced extract.

3.2.3.2. mCry3A characterisation and equivalence[15]

SDS-PAGE and Western blot analysis showed that plant- and microbe-derived mCry3A proteins had the expected molecular weight of ~ 67.7 kDa and were comparably immunoreactive to antibodies capable of detecting mCry3A protein. Glycosylation detection analysis demonstrated that none of the mCry3A proteins were glycosylated. Amino acid sequence analysis by mass spectrometry methods showed that both proteins matched the deduced sequence as defined by the *mCry3A* gene.

3.2.3.3. PAT characterisation and equivalence

SDS-PAGE and Western blot analysis showed that plant- and microbe-derived PAT proteins had the expected molecular weight of ~ 20.5 kDa and were comparably immunoreactive to PAT-specific antibodies. Glycosylation detection analysis demonstrated that none of the PAT proteins were glycosylated. Amino acid sequence analysis by mass spectrometry methods showed that both proteins matched the deduced sequence as defined by the *PAT* gene. These data also showed that the N-terminal methionine of the plant-produced PAT protein was truncated. Such modifications are common in eukaryotic proteins (e.g. Polevoda and Sherman, 2000). Functional equivalence was demonstrated by a biochemical *in vitro* activity assay which showed that both proteins had comparable activity for the intended herbicide.

The protein characterisation data comparing the structural, biochemical and functional properties of plant and *E. coli*-produced eCry3.1Ab, mCry3A and PAT proteins indicate that these proteins are equivalent. Therefore, the GMO Panel accepts the use of the eCry3.1Ab, mCry3A and PAT proteins produced in bacteria for the safety studies.

3.2.4. Information on the expression of the insert[15]

Protein levels of eCry3.1Ab, mCry3A and PAT were analysed by enzyme-linked immunosorbent assay (ELISA) in material harvested in a field trial across four locations in the US during the 2013 growing season. Samples analysed included leaves (V6, R1, R6 and senescence), roots (V6, R1, R6 and senescence), whole plants (V6, R1 and R6), pollen (R1) and grain (R6 and senescence) from both those treated and not treated with glufosinate. The mean values, standard deviations and ranges of protein expression levels in grain (R6 and senescence, n = 20), whole plant (R6, n = 20) and pollen (R1, n = 4) of the eCry3.1Ab, mCry3A and PAT proteins used to estimate human and animal dietary exposure (see Section 3.4.5) are reported in Table 1.

[14] Dossier: Part II – Section 1.2.2.3 and additional information: 20/6/2018; 30/4/2020.
[15] Dossier: Part II - Section 1.2.2.3 and additional information 20/6/2018.

Table 1: Mean values, standard deviations and ranges of newly expressed proteins in grain (n = 20), whole plant (n = 20) and pollen (n = 4) [μg/g dry weight (dw)/μg/g fresh weight (fw)] from maize MZIR098

	Glufosinate treatment			
	Untreated		Treated	
	μg/g dry weight (dw)	μg/g fresh weight (fw)	μg/g dry weight (dw)	μg/g fresh weight (fw)
Grain (R6)				
eCry3.1Ab	2.42[(a)] ± 1.15[(b)] (1.28–5.90)[(c)]	1.58 ± 0.62 (1.02–3.44)	1.88 ± 1.01 (0.76–4.84)	1.23 ± 0.58 (0.56–2.94)
mCry3A	14.59 ± 3.76 (8.91–22.83)	9.76 ± 2.36 (6.05–15.38)	14.51 ± 3.37 (8.12–22.38)	9.73 ± 2.27 (5.98–15.75)
PAT	– (< LOD – < LOQ)[(d)]	– (< LOD – < LOQ)[(d)]	– (< LOD)[(d)]	– (< LOD)[(d)]
Grain (senescence)				
eCry3.1Ab	2.08 ± 1.29 (0.82–4.52)	1.50 ± 0.79 (0.67–3.03)	1.94 ± 0.90 (0.76–3.72)	1.42 ± 0.56 (0.63–2.62)
mCry3A	11.21 ± 3.41 (6.69–19.65)	8.30 ± 2.02 (5.38–14.14)	10.12 ± 2.58 (5.77–14.51)	7.55 ± 1.58 (4.63–10.34)
PAT	– (< LOD)[(d)]	– (< LOD)[(d)]	– (< LOD)[(d)]	– (< LOD)[(d)]
Whole plant (R6)				
eCry3.1Ab	7.72 ± 3.48 (2.70–18.38)	3.47 ± 1.0 (1.57–6.47)	7.97 ± 3.73 (2.91–15.07)	3.64 ± 1.45 (1.73–6.48)
mCry3A	14.49 ± 4.29 (9.20–26.36)	6.87 ± 2.31 (3.48–12.62)	16.06 ± 6.32 (8.13–30.24)	7.77 ± 3.43 (3.85–14.53)
PAT	– (< LOD – 0.36)[(d)]	– (< LOD – 0.14)[(d)]	– (< LOD – 0.37)[(d)]	– (< LOD – 0.18)[(d)]
Pollen (R1)				
eCry3.1Ab	– (< LOD)[(d)]	– (< LOD)[(d)]	– (< LOD)[(d)]	– (< LOD)[(d)]
mCry3A	302.93 ± 6.37 (293.87–308.71)	187.75 ± 41.14 (152.64–246.96)	312.48 ± 15.84 (289.93–326.92)	183.91 ± 37.28 (154.06–236.71)
PAT	– (< LOD)[(d)]	– (< LOD)[(d)]	– (< LOD)[(d)]	– (< LOD)[(d)]

–: Not applicable.
(a): Average.
(b): Standard deviation.
(c): Range.
(d): LOD for eCry3.1Ab = 0.08 μg/g dw; LOD for PAT = 0.025 μg/g dw; LOQ for PAT = 0.031 μg/g dw.

3.2.5. Inheritance and stability of inserted DNA[16]

Genetic stability of the maize MZIR098 insert was assessed by Southern analysis of genomic DNA from five generations.[17] The restriction enzyme/probe combinations used 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.

Phenotypic stability was assessed by measuring concentration of eCry3.1Ab, mCry3A and PAT proteins in leaves, root, pollen and kernels, collected from three generations. The expression of the eCry3.1Ab, mCry3A and PAT proteins was confirmed in the tested tissues, except for eCry3.1Ab in pollen and PAT in pollen and kernels.[18] The inheritance pattern was investigated by PCR, using

[16] Dossier: Part II - Section 1.2.2.4.
[17] Appendix 1.2.12.
[18] Appendix 1.2.13.

ecry3.1Ab, *mcry3A* and *pat* gene-specific probes; the results supported the presence of a single insertion, segregating in a Mendelian fashion.[19]

3.2.6. Conclusion on molecular characterisation

The molecular characterisation data establish that maize MZIR098 contains a single insert consisting of one copy of the eCry3.1Ab, mCry3A and PAT expression cassettes. Bioinformatics analyses of the sequences encoding the newly expressed proteins and ORFs within the insert or spanning the junctions between the insert and genomic DNA do not raise concerns that need additional food/feed safety considerations. The stability of the inserted DNA and of the introduced traits is confirmed over several generations. The methodology used to quantify the levels of the eCry3.1Ab, mCry3A and PAT proteins is considered adequate. The protein characterisation data comparing the structural and biochemical properties of plant- and microbe-derived eCry3.1Ab, mCry3A and PAT proteins indicate that these proteins are equivalent, and the microbe-produced protein can be used in safety studies.

3.3. Comparative analysis[20]

3.3.1. Overview of studies conducted for the comparative analysis

Application EFSA-GMO-DE-2017-142 presents data on agronomic/phenotypic characteristics as well as on forage and grain composition of maize MZIR098. In addition, the application contains data on characteristics of seed from maize MZIR098 (Table 2).

Table 2: Main comparative analysis studies to characterise maize MZIR098 provided in application EFSA-GMO-DE-2017-142

Study focus	Study details	Comparator	Commercial non-GM reference varieties
Agronomic and phenotypic analysis	Field study, USA, 2013, 9 sites(a)	NP2222 × NP2391	6(b)
Compositional analysis	Field study, USA, 2013, 8 sites(a)		
Seed germination	F_1 grains tested under controlled conditions		3(c)

(a): The field trials were located in Richland, IA; Bagley, IA; Seymour, IL; Wyoming, IL; Stewardson, IL; Larned, KS; York, NE; and Germansville, PA. A field trial established in Carlyle, IL in 2013 was removed from the study due to unfavourable weather conditions resulting on stand failure. An additional field trial established in Delavan, WI in 2013 was partially removed from the study, as weather did not support normal maturity and yield, therefore not included for the compositional analysis.
(b): Non-GM hybrid maize used in the field studies were: H-7191, H-7540, SY Generoso, NK Lucius, Cisko, SY Provial.
(c): Non-GM hybrid maize were: NK Octet, NK Lucius and Cisko.

3.3.2. Experimental field trial design and statistical analysis

At each field trial site, the following materials were grown: maize MZIR098, the comparator maize NP2222 × NP2391 and six commercial non-GM maize reference varieties, all treated with conventional herbicides management regimes; and maize MZIR098 exposed to the intended glufosinate-ammonium-containing herbicide, in addition to the conventional herbicides.

The agronomic/phenotypic and compositional data were analysed as specified by the GMO Panel (EFSA GMO Panel, 2010b, 2011a). This includes, for each of the two treatments of maize MZIR098, the application of a difference test (between the GM maize and its comparator) and an equivalence test (between the GM maize and the set of non-GM maize reference varieties).[21] The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).

[19] Appendix 1.2.6.
[20] Dossier: Part II – Section 7; Additional information: 27/10/2017, 20/4/2018 and 20/6/2018.
[21] The purpose of the test of equivalence is to evaluate the estimated mean values for maize MZIR098 taking into account natural variability as defined by a set of commercial non-GM maize reference varieties with a history of safe use for consumption as food or feed.

3.3.3. Suitability of selected test materials

3.3.3.1. Selection of the GM maize line and comparator

Inbred line NP2222 was transformed to obtain MZIR098 which then crossed with the inbred line NP2391 to produce the hybrid maize MZIR098 used in the comparative analysis.

The comparator used in the field trials is the non-GM maize hybrid NP2391 × NP2222, which has the same genetic background as maize MZIR098 (as documented by the pedigree) and is therefore considered to be the conventional counterpart.

Maize MZIR098 and its conventional counterpart have a comparative relative maturity (CRM) ranging between 105 and 107 and are suitable for growing in a range of environments across North America.

3.3.3.2. Selection of commercial non-GM maize reference varieties

Six commercial non-GM maize reference varieties with a CRM ranging from 93 to 115 were grown at each field trial site (see Table 2). Based on the information on the relative maturity classes, the GMO Panel considers that the selected non-GM maize reference varieties are appropriate for the comparative analysis.

3.3.3.3 Seed production and quality

Seeds of maize MZIR098 and its conventional counterpart used in the field trials (see Table 2) were produced, harvested and stored under similar conditions. The genetic purity of maize MZIR098 seed lots was confirmed via event-specific real-time PCR analysis.

The applicant tested the germination rate of seeds from maize MZIR098 (F_1 grains), the conventional counterpart and three non-GM maize varieties (see Table 2). Germination was tested in growth chambers under controlled conditions at six different temperature regimes.[22] The endpoints analysed were the numbers of normal germinated seeds, abnormal germinated seeds, dead seeds and dormant and hard seeds.

No statistically significant differences were observed between the germination rates of GM maize MZIR098 compared to its conventional counterpart.

The test materials used in the seed germination study derive from seed lots other than the one used for the field trials. Therefore, the GMO Panel considers that the study does not allow drawing conclusions on the specific germinability of the test materials used for the comparative analysis, but only on possible unintended effects due to the presence of event MZIR098.

Although the applicant refers to seed dormancy when discussing the generated data on seed characteristics of maize MZIR098, the EFSA GMO Panel considered that only the conclusions on seed germination of maize MZIR098 are substantiated by the provided data.

3.3.3.4. Conclusion on suitability

The GMO Panel is of the opinion that the maize MZIR098 hybrid, its conventional counterpart and the non-GM maize reference varieties were properly selected and are of adequate quality. Therefore, the test materials are considered suitable for the comparative analysis.

3.3.4. Representativeness of the receiving environments

3.3.4.1 Selection of field trial sites

The sites selected for the field trials were located in commercial maize-growing regions of North America. The climate and soil characteristics of the selected sites were diverse,[23] corresponding to optimal near-optimal and suboptimal conditions for the cultivation of maize (Sys et al., 1993). The GMO Panel considers that the selected sites reflect commercial maize-growing regions in which the test materials are likely to be grown.

3.3.4.2. Meteorological conditions

Maximum and minimum mean temperatures and sum of precipitations were provided on a monthly basis. No exceptional weather conditions were reported at any of the selected sites. The GMO

[22] Constant temperatures at 10°C, 25°C and 30°C plus alternating temperatures 10°C for 16 h and 20°C for 8 h at 10°C, 10°C and 30°C, and 20°C and 30°C.

[23] Soil types of the field trials were silty clay loam, clay loam, loam and silt loam. Mean temperatures and sum of precipitations during the usual maize-growing season ranged, respectively, from 15.8°C to 22.2°C and from 364 mm to 772 mm.

Panel considers that the meteorological data set falls within the range of climatic conditions normally occurring at these sites.

3.3.4.3. Management practices

The field trials included plots containing maize MZIR098, plots with the conventional counterpart and plots with non-GM reference varieties, treated with conventional herbicide management regimes. In addition, the field trials included plots containing maize MZIR098 managed following the same agricultural practices, plus exposed to glufosinate-ammonium-containing herbicide, applied at the BBCH 13–14 growth stage.

At some field trial sites, sowing occurred later than usual, resulting in a shorter growing cycle. The applicant provided information indicating that the shorter growing cycle was unlikely to affect the agronomic/phenotypic and compositional data. In addition, thinning was applied at all field trials to achieve a more homogeneous plant density across plots.

Despite late sowing and thinning represent deviations from standard management practices under farm cultivation, those agronomic practices do not alter the capability to conclude on the comparative assessment. Therefore, the GMO Panel considers that the management practices including sowing, harvesting and application of plant protection products were acceptable.

3.3.4.4. Conclusion on representativeness

The GMO Panel concludes that the geographical locations, climate and soil characteristics, meteorological conditions and most of the management practices of the field trials are typical for receiving environments where the tested materials could be grown.

3.3.5. Agronomic and phenotypic analysis

3.3.5.1. Agronomic and phenotypic endpoints tested under field conditions

Eleven agronomic and phenotypic endpoints[24] plus information on abiotic stressors, disease incidence and arthropod damage were collected from the field trials (see Table 2). Two endpoints (early stand count after thinning and total lodging[25]) were not subjected to a formal statistical analysis (Section 3.3.2) because the data did not fulfil the assumptions of analysis of variance.

The test of difference and the test of equivalence were applied to eight endpoints, with the following results:

- For maize MZIR098 (not treated), the test of difference identified no statistically significant differences with the conventional counterpart.
- For maize MZIR098 (treated), statistically significant differences were identified for days to 50% pollen shed and days to 50% silking. Both endpoints fell under equivalence category I.

3.3.6. Compositional analysis

Forage and grain harvested from the field trials in the US in 2013 (see Table 2) were analysed for 81 different constituents (9 in forage and 72 in grain), including the key constituents recommended by the OECD (OECD, 2002). For 15 grain components, a large part of the observations was below the limit of quantification.[26] The statistical analysis was applied to the remaining 66 constituents (9 in forage[27] and 57 in grain[28]); a summary of the outcome of the test of difference and the test of equivalence is presented in Table 3.

[24] Early stand count, thinned stand count, final stand count, days to 50% pollen shed, days to 50% silking, plant height, stalk lodged plants, root lodged plants, grain moisture, grain test weight and grain yield.

[25] The applicant reported lodging as a total representing the sum of root- and stalk-lodging.

[26] Selenium, 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); pentadecanoic acid (C15:1); heptadecenoic acid (C17:1), γ-linolenic acid (C18:3), eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3) and arachidonic acid (C20:4).

[27] Ash, moisture, carbohydrates, fat, protein, calcium, phosphorus, acid detergent fibre (ADF) and neutral detergent fibre (NDF).

[28] Ash, carbohydrates, fat, protein, starch, acid detergent fibre (ADF), neutral detergent fibre (NDF), total dietary fibre (TDF), calcium, copper, iron, magnesium, manganese, phosphorus, potassium, zinc, alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C21:0), behenic acid (C22:0), α-tocopherol, β-carotene, folic acid, niacin, pyridoxine, riboflavin, thiamine ferulic acid, *p*-coumaric acid, inositol, phytic acid, raffinose and trypsin inhibitor.

- For maize MZIR098 (not treated), statistically significant differences with the conventional counterpart were identified for 11 endpoints (one in forage and 10 in grain), which all fell under equivalence category I or II.
- For maize MZIR098 (treated), statistically significant differences with the conventional counterpart were identified for 12 endpoints (one in forage and 11 in grain), which all fell under equivalence category I or II except for NDF in grain (Table 4).

Table 3: Summary of the outcome of the comparative analysis in grain and forage from maize MZIR098. The table shows the number of endpoints in each category

		Test of difference[a]			
		Not treated[c]		Treated	
		Not different	Significantly different	Not different	Significantly different
Test of equivalence[b]	Category I/II	53	11[d]	53	11[d]
	Category III/IV	2[e]	–	1[e]	1[f]
	Total endpoints	66			66

(a): Comparison between the GM maize and its conventional counterpart.
(b): Four different outcomes: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence).
(c): Treated/not treated with the intended herbicide (glufosinate-ammonium).
(d): Endpoints with significant differences between the GM maize and its conventional counterpart and falling in equivalence category I-II. In forage, not treated only: phosphorus. Treated only: fat. In grain, not treated only: lysine, calcium, copper and zinc. Treated only: stearic acid (C18:0), phosphorus, starch and α-tocopherol. Both treated and not treated: arachidic acid (C20:0), heptadecanoic acid (C17:0), linoleic acid (C18:2), oleic acid (C18:1), potassium and β-carotene.
(e): Endpoints falling in equivalence category III–IV and with no significant differences between the GM maize and its conventional counterpart. In forage, none. In grain, not treated only: ADF. Both treated and not treated: ferulic acid.
(f): NDF in grain was significantly different between the GM maize (not treated) and its conventional counterpart and fell under equivalence category III. Quantitative results for NDF in grain are reported in Table 4.

The GMO Panel assessed all the significant differences between maize MZIR098 and its conventional counterpart, taking into account the potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties. Quantitative results for the endpoint showing a significant difference between maize MZIR098 and its conventional counterpart and falling under equivalence category III are given in Table 4.

Table 4: Quantitative results (estimated means and equivalence limits) for the endpoint with a significant difference between maize MZIR098 and its conventional counterpart and falling under equivalence category III (see Table 3)

	Endpoint	Maize MZIR098		Conventional counterpart	Non-GM reference varieties	
		Not treated	Treated[a]		Mean	Equivalence limits
Grain	NDF (% DM)	11.3	11.5*	11.1	9.5	7.9–11.4

DM: dry matter.
(a): Not treated: treated only with conventional herbicides. Treated: treated with the intended herbicide glufosinate ammonium.
For the GM maize, significantly different values are marked with an asterisk, while the outcomes of the test of equivalence are differentiated by greyscale backgrounds: the light grey background corresponds to equivalence category III and the white background to equivalence category II.
Means and equivalence limits were calculated on a log-transformed scale; the values shown in the table are back-transformed to the original scale.

3.3.7. Conclusion on the comparative analysis

Taking into account the natural variability observed for the set of non-GM reference varieties, the GMO Panel concludes that:

- None of the differences identified in the agronomic and phenotypic characteristics tested between maize MZIR098 and its conventional counterpart needs further assessment for potential environmental impact.
- None of the compositional differences between maize MZIR098 and its conventional counterpart needs further assessment for food/feed safety except for NDF in grain treated with the intended herbicide, which is discussed in Sections 3.4.3.3 and 3.4.7.

3.4. Food/feed safety assessment

3.4.1. Effects of processing[29]

Maize MZIR098 will undergo existing production processes used for conventional maize. Considering the changes observed in the compositional comparative analysis (Section 3.3.6), the processing of maize MZIR098 into food and feed products is not expected to result in products being different from those of commercial non-GM maize varieties.

3.4.2. Influence of temperature and pH on newly expressed proteins[30]

Effects of temperature and pH on the newly expressed proteins in this GM maize have been previously evaluated by the GMO Panel (EFSA GMO Panel, 2018a,b; EFSA 2009a,b). Additional studies were provided by the applicant (Appendix A). The outcome of these studies is consistent with similar studies previous assessed by the GMO Panel.

3.4.3. Toxicology

3.4.3.1. Testing of newly expressed proteins[31]

The three proteins newly expressed in maize MZIR098 (eCry3.1Ab, mCry3A and PAT) have been extensively characterised and their equivalence to *E. coli*-produced proteins used in safety studies was demonstrated (Section 3.2.3).

The eCry3.1Ab, mCry3A and PAT proteins were previously assessed by the GMO Panel in the context of other applications (i.e. EFSA GMO Panel, 2018a,b; EFSA 2009a,b) and no safety concerns for humans and animals were identified.

Updated bioinformatics analyses revealed no similarities of the eCry3.1Ab, mCry3A and PAT proteins with known toxins.

Additional studies addressing *in vitro* degradation of the newly expressed proteins were provided by the applicant (Appendix A). The outcome of these studies is consistent with previous studies assessed by the GMO Panel (EFSA GMO Panel, 2018a,b; EFSA 2009a,b).

Additional studies addressing acute and subacute toxicity of the eCry3.1Ab, mCry3A and PAT proteins were provided by the applicant and assessed by the GMO Panel (Appendix A).

The GMO Panel is not aware of any new information that would change previous conclusion on the safety of the eCry3.1Ab, mCry3A and PAT proteins.

Based on scientific knowledge, no synergistic or antagonistic interactions raising food/feed safety concerns exist between the eCry3.1Ab, mCry3A and PAT proteins.

3.4.3.2. Testing of new constituents other than newly expressed proteins

No new constituents other than newly expressed proteins have been identified in grain and forage from maize MZIR098. Therefore, no further food/feed safety assessment of components other than the newly expressed proteins is required.

3.4.3.3. Information on altered levels of food and feed constituents

Neutral detergent fibre (NDF) levels in grains were significantly different in maize MZIR098 treated with the intended herbicide when compared with its conventional counterpart and showed a lack of equivalence with the non-GM reference varieties (Section 3.3.6). Taking into account the biological characteristics and functions of this compound, the observed difference is considered of no toxicological concern. Further information on safety is provided in Section 3.4.7.

[29] Dossier: Part II – Section 1.3.6
[30] Dossier: Part II – Section 1.5.1, Section 7 and Appendix A.
[31] Dossier: Part II – Section 1.4.1.

3.4.3.4. Testing of the whole genetically modified food and feed[32]

Based on the outcome of the studies considered in the molecular characterisation and comparative analysis, no compositional modifications, or indication of possible unintended effects relevant to food/feed safety of maize MZIR098 have been identified. Therefore, animal feeding studies with food/feed derived from maize MZIR098 are not considered necessary by the GMO Panel (EFSA GMO Panel, 2011a). In accordance with Regulation (EU) No 503/2013, the applicant provided a 90-day feeding study in rats receiving diets derived from MZIR098 maize (study number 501204). The applicant provided spontaneously a second 90-day feeding study in rats (study number 503615) receiving diets derived from MZIR098 maize, which was considered by the GMO Panel.[33]

In each study, pair-housed Han Wistar rats (RccHan:WIST) (10 per sex per group; 2 rats per cage) were allocated to four groups using a randomised complete block design with five replications per sex. Groups were fed test or control diets containing 10% or 41.5% (w/w) maize from maize MZIR098 plants treated with the intended herbicide glufosinate ammonium (test material), or from the conventional counterpart (control material), respectively. The studies were adapted from OECD test guideline 408 (1998), aligned with the guidance of the EFSA Scientific Committee (2011) and comply with the principles of good laboratory practice (GLP) with some minor deviations not impacting the study results and interpretation (i.e. test item stability, homogeneity and concentration), which are detailed below. Event-specific PCR analysis confirmed the presence of the event MZIR098 in the GM maize and GM diets and excluded the presence of the event in the respective controls. ELISA analyses also confirm the presence of event MZIR098 in the GM maize and GM diets. Both GM and control diets were analysed for nutrients, antinutrients and potential contaminants (e.g. selected heavy metals, mycotoxins, pesticides and microorganisms). Balanced diets were based on the CT1 diet prepared by Special Diet Services. The stability of the test and control materials was not verified in the studies for the duration of the treatment; however, in accordance to product expiration declared by the diet manufacturer, the constituents of the diets are considered stable. The GMO Panel considered this justification acceptable. Diet preparation procedures and regular evaluations of the mixing methods guaranteed the homogeneity and the proper concentration of the test or control substances in them. Feed and water were provided *ad libitum*. In-life procedures and observations and terminal procedures were conducted in accordance to OECD TG 408 (1998). In the statistical analysis, for each of the two inclusion rates, rats consuming the test diet were compared with those consuming the respective control diet.[34] The cage was considered the experimental unit. For continuous parameters, a multi-way analysis of variance (ANOVA) was conducted for the two sexes combined (factors: treatment, sex, block-within-sex and sex-by-treatment interaction); in case a significant sex-by-treatment interaction was identified, a two-way ANOVA (factors: treatment and block) was performed separately for males and females. The two-way ANOVA was also used to analyse sex-specific organ weights.

Intakes of MZIR098 in study 501204 were 6.8 g/kg bw per d and 29.3 g/kg bw per d in the 10% and 41.5% males, respectively, and 8.4 g/kg bw per d and 33.2 g/kg bw per d in the 10% and 41.5% females, respectively. Intakes of MZIR098 in study 503615 were 7.5 g/kg bw per d and 30.1 g/kg bw per d in the 10% and 41.5% males, respectively, and 8.8 g/kg bw per d and 35.7 g/kg bw per d in the 10% and 41.5% females, respectively. All animals survived the treatment period. No test diet-related clinical signs or ophthalmoscopic findings are observed. There were no treatment-related findings reported in the gross or histopathological examinations and the pattern of findings were consistent with those of rats of this strain and age. Statistically significant results were identified in different endpoints[35] ; however, these were considered either not to be adverse or to be spontaneous in nature for one or more of the following reasons:

- The finding was present only at the 10% incorporation rate but not in the 41.5% group;
- The magnitude of the change was small (e.g. < 10%) and of no impact on the physiology of the rats;
- The changes were within the normal range of variation seen for the parameter;

[32] Dossier: Part II – Section 1.4.4 and Additional information: 27/10/2017.

[33] The two studies provided were identical in all key aspects other than the number of rats sampled for clinical pathology; 7 males and 9 or 10 females per group in the study originally submitted in the dossier and all 10 rats per group in the study provided spontaneously.

[34] There was a difference in the preparation of the 10% and 41.5% diets, which prevented a statistical comparison using both groups.

[35] Body weights and cumulative body weight gain, food consumption and utilisation, haematology and clinical chemistry, organ weights, detailed functional observation.

- The changes were significant only at one or two intermediate time points but not over the whole duration of the investigation;
- There was no consistency of findings within a study (e.g. organ weight changes with no related histopathology findings);
- There were no consistent patterns across the two essentially identical studies.

The GMO Panel concludes that the incorporation of maize MZIR098 into the diet of rats at 10% or 41.5% did not produce any adverse effects in either study 501204 or 503615. The GMO Panel notes that the applicant only tested 41.5% dose level with the full set of OECD parameters; this incorporation rate of maize is in line with commercially available rodent diets. It has been recently reported that a diet incorporating 50% maize may be tolerated without inducing nutritional imbalances in rats after 90-day administration (Steinberg et al., 2019), but the GMO Panel considers that further scientific confirmation is needed before this 50% maize incorporation rate is applicable in future studies.

3.4.4. Allergenicity

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

3.4.4.1. Assessment of allergenicity of the newly expressed proteins[36]

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

The *ecry3.1Ab* and *mcry3A* genes originate from *B. thuringiensis*, while the *pat* gene originates from *S. viridochromogenes*, none of which are considered allergenic sources.

Updated bioinformatic analyses of the amino acid sequences of the eCry3.1Ab, mCry3A and PAT proteins, using the criterion of 35% identity in a sliding window of 80 amino acids, revealed no significant similarities to known allergens. In addition, the applicant also performed analyses searching for matches of eight contiguous identical amino acid sequences between these newly expressed proteins and known allergens, which confirmed the outcome of the previous bioinformatic analyses. The studies on resistance of the eCry3.1Ab, mCry3A and PAT proteins to degradation by pepsin have been described in Section 3.4.3.1.

The GMO Panel has previously evaluated the safety of the eCry3.1Ab, mCry3A and PAT proteins and no concerns on allergenicity were identified (EFSA GMO Panel, 2018a,b; EFSA 2009a,b). Based on current knowledge, and as there is no evidence of allergenicity of the newly expressed proteins, there are no expected concerns of allergenicity as a consequence of their interaction in this GM maize.

Furthermore, no concerns on adjuvanticity of the eCry3.1Ab, mCry3A and PAT proteins were identified in the context of the applications assessed (EFSA GMO Panel, 2018a,b; EFSA 2009a,b). The GMO Panel did not find indications that the Bt proteins at the levels expressed in this GM maize might be adjuvants able to enhance an allergic reaction.

In the context of this application, the GMO Panel considers that there are no indications that the newly expressed eCry3.1Ab, mCry3A and/or PAT proteins in maize MZIR098 may be allergenic.

3.4.4.2. Assessment of allergenicity of the whole GM plant[37]

The EFSA GMO Panel regularly reviews the available publications on food allergy to maize. However, maize is not considered a common allergenic food (OECD, 2002).[38] Therefore, the EFSA GMO Panel does not request experimental data to analyse the allergen repertoire of GM maize.

[36] Dossier: Part II - Section 1.5.1 and 1.5.3.
[37] Dossier: Part II - Section 1.5.2., Section 7 and Appendix A.
[38] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

In the context of this application and considering the data from the molecular characterisation, the compositional analysis and the assessment of the newly expressed proteins (see Sections 3.2, 3.3 and 3.4), the GMO Panel identified no indications of a potentially increased allergenicity of food and feed derived from the GM maize MZIR098 with respect to that derived from the non-GM conventional counterpart.

3.4.5. Dietary exposure assessment to new constituents

In line with Regulation (EU) No 503/2013, the applicant provided dietary exposure estimates to eCry3.1Ab, mCry3A and PAT proteins which are newly expressed in maize MZIR098. Dietary exposure was estimated based on protein expression levels reported in this application for maize MZIR098 treated with the intended herbicide, the current available consumption data and feed practices, the foods and feeds currently available in the market and the described processing conditions. Table 1 in Section 3.2.4. shows the protein expression levels used to estimate both human and animal dietary exposure.

3.4.5.1. Human dietary exposure[39]

As per request of the GMO Panel, chronic and acute dietary exposure to the newly expressed proteins in MZIR098 maize grains was provided. The applicant followed the methodology described by EFSA to estimate dietary exposure in high consumers using summary statistics (EFSA, 2015).

Human dietary exposure was estimated across different European countries on different population groups: young population (infants, toddlers, 'Other children'), adolescents, adult population (adults, elderly and very elderly) and special populations (pregnant and lactating women). Since no specific consumption data were available on commodities containing, consisting of or obtained from MZIR098 maize grains, a conservative scenario with 100% replacement of conventional maize by the GM maize was considered. Consumption figures for all relevant commodities (e.g. corn flakes, sweet corn, popcorn, etc.) were retrieved from the EFSA Comprehensive European Food Consumption Database (EFSA consumption database).[40] Corn oil was excluded from the assessment since no proteins are expected to be present in the oil.

Mean protein expression values on fresh weight basis (Table 1) are considered as the most adequate to estimate human dietary exposure (both acute and chronic) when working with raw primary commodities that are commonly consumed as processed blended commodities (EFSA, 2019). For PAT protein, the limit of detection (LOD = 0.025 μg/g) was used as worst-case scenario for the exposure estimations as all analytical measurements were below the LOD. The protein content in the different maize processed commodities was used to estimate the concentration of the newly expressed proteins in the consumed foods.[41] This is a conservative approach as neither recipes nor the effect of processing is considered on the final concentration of newly expressed proteins, except for corn oil which is eventually excluded from the exposure estimations.

The highest acute dietary exposure was estimated in the age class 'Other children' with exposure estimates of 27 μg/kg bw day, 145 μg/kg bw day and 0.5 μg/kg bw day for eCry3.1Ab, mCry3A and PAT proteins, respectively. The main average contributor to the exposure in the dietary survey with the highest estimates was popcorn.

The highest chronic dietary exposure was estimated in the age class 'Toddlers' with exposure estimates of 11 μg/kg bw day, 59 μg/kg bw day and 0.2 μg/kg bw day for eCry3.1Ab, mCry3A and PAT proteins, respectively. The main average contributor to the exposure in the dietary survey with the highest estimates was corn chips.

An ad hoc dietary exposure scenario was carried out for consumers of pollen supplements under the assumption that these supplements might be made of pollen from MZIR098 maize. From the expression values reported in Table 1 in pollen, the concentrations of eCry3.1Ab, mCry3A and PAT proteins in pollen supplements were calculated, assuming around 6% moisture content. For eCry3.1Ab, and PAT proteins, the reported LODs were used to estimate dietary exposure since no samples reported detected levels of these proteins in pollen. Consumption data on pollen supplements are available for few consumers across nine different European countries[42]; the low number of consumers available adds uncertainty to the exposure estimations and prevent from estimate exposure for high

[39] Dossier: Part II – Section 2.A; additional information 20/6/2018, 29/11/2018, 30/4/2020.
[40] http://www.efsa.europa.eu/en/data/food-consumption-data. Data accessed on April 2018.
[41] Median protein values were derived using different national food composition databases.
[42] https://www.efsa.europa.eu/en/food-consumption/comprehensive-database. Data accessed April 2020.

consumers of pollen supplements. Among consumers of pollen supplements, the highest average acute dietary exposure estimates would range from 0.02 μg/kg bw per day for PAT to 218 μg/kg bw per day for mCry3A, in the elderly population. Similarly, the highest average chronic dietary exposure estimates would range from 0.01 μg/kg bw per day for PAT to 145 μg/kg bw per day for mCry3A, also in the elderly population.

3.4.5.2. Animal dietary exposure[43]

Dietary exposure to eCry3.1Ab, mCry3A and PAT proteins in maize MZIR098 was estimated across different livestock animal species as described below, assuming the consumption of maize products commonly entering the feed supply chain (i.e. grains, milled by products, hominy, gluten feed, gluten meal, forage and stover). A conservative scenario with 100% replacement of conventional maize products by the maize MZIR098 products was considered.

Mean levels on fresh weight basis of eCry3.1Ab, mCry3A and PAT proteins in grain and forage (whole plant) from maize MZIR098 treated with the intended herbicide used for animal dietary exposure are listed in Table 1. For PAT protein, the limit of detection (LOD = 0.022 μg/g FW in senescence grains and 0.053 μg/g FW in whole plant R6) was used as worst-case scenario for the exposure estimations as some analytical measurements[44] were below the LOD or the limit of quantification (LOQ). To estimate the mean levels of eCry3.1Ab, mCry3A and PAT proteins in maize by-products and stover, factors were applied[45] based on the protein content in these feed fractions relative to maize grain and forage, respectively, and assuming that no losses of newly expressed proteins occur during processing.

The applicant estimated dietary exposure to eCry3.1Ab, mCry3A and PAT proteins in livestock (e.g. cattle, sheep, swine and poultry) based on estimates for body weights, daily feed intakes and inclusion rates (percentages) of maize grain and by-products (i.e. milled by products, hominy, gluten feed, gluten meal), forage and stover in diets/rations (OECD, 2013).

The theoretical maximum contribution to the highest exposure to eCry3.1Ab, mCry3A and PAT proteins was taken into account for each feedstuff.[46]

Estimated dietary exposure is reported in Table 5.

Table 5: Dietary exposure to eCry3.1Ab, mCry3A and PAT proteins (mg/kg bw per day) in livestock

	Dietary exposure (mg/kg bw per day)		
	eCry3.1Ab	**mCry3A**	**PAT**
Beef cattle	0.258	0.817	0.0038
Dairy cattle	0.397	1.44	0.0060
Ram/Ewes	0.243	1.29	0.0038
Lambs	0.426	1.89	0.0065
Breeding swine	0.120	0.506	0.0018
Finishing swine	0.102	0.542	0.0016
Broiler hens	0.240	1.27	0.0037
Laying hens	0.295	1.37	0.0045
Turkey	0.219	1.17	0.0034

3.4.6. Nutritional assessment of endogenous constituents

The intended traits of maize MZIR098 are herbicide tolerance and insect resistance, with no intention to alter nutritional parameters. However, neutral detergent fibre (NDF) in treated grains was significantly different from its conventional counterpart and showed a lack of equivalence with the set of non-GM reference varieties (Section 3.3.6). The biological relevance of NDF, the role of maize as contributor to its total intake and the magnitude and direction of the observed change were considered during the nutritional assessment.

[43] Dossier: Part II – Section 2.B; additional information 22/1/2020, 30/4/2020.
[44] All 20 observations were < LOD in senescence grains; nine samples were either < LOD or < LOQ in whole plant (R6).
[45] eCry3.1Ab, mCry3A and PAT proteins concentration for maize by-products and stover GM was not available. Therefore, protein concentration was normalised to grain using the following factors (rounded to two decimal places): hominy 1.18, milled by-products 0.90, gluten feed 2.13 and gluten meal 6.38. Protein concentration in stover was normalised to forage by using a factor of 0.86.
[46] Description of the model applied was provided in the study report #SSB-179-17 A2.

3.4.6.1. Human nutrition

In the context of human nutrition, fibre is referred to as dietary fibre, which primarily includes non-starch polysaccharides (mainly cellulose, hemicelluloses, pectins and other hydrocolloids) and lignin (EFSA NDA Panel, 2010). Consequently, the minor observed increase (~ 4%) in NDF (lignin, hemicellulose and cellulose) implies an increased intake of dietary fibre. No Tolerable Upper Intake Level (UL) is derived for dietary fibre and, on contrary, there are nutritional recommendations to increase its intake levels based on its key role on bowel function (EFSA NDA Panel, 2010). Based on this, the GMO Panel considers that the observed increase in NDF in maize MZIR098 does not represent any nutritional concern in humans.

3.4.6.2. Animal Nutrition

In the context of animal nutrition, neutral detergent fibre (NDF) can be regarded as a measure of the plant cell wall material, consisting mainly of lignin, cellulose and hemicellulose. Ruminant's diet consists in particularly of plants and their by-products containing variable amounts of these high undegradable fibres, used as energy source by rumen microbes. The limiting factor of fibre digestibility in ruminant is excessive presence of lignin, which makes cellulose and hemicellulose less available by combining with them. However, the minimal differences observed with the conventional counterpart (~ 4%) and the reference varieties of the total NDF do not have a biological significance.

In contrast, monogastric animals cannot use the fibres as energy source, because they lack gastric bacterial fermentation and do not have endogenous enzyme capable to digest fibre. However, up to certain amount, these fibres can be digested by microbes present in the large intestine. Even in monogastric animals, the minimal differences observed with the conventional counterpart (~ 4%) and the reference varieties do not have a biological significance.

In addition, it is noted that international feed databases (e.g. 'Feed Tables' (https://www.feedtables.com/content/maize) report variability in maize grain NDF mean values, the average value is reported to be 12.5% DM, with an SD of 1.6 and the range is from 7.7% to 16.5% DM, data based on 149 counts.

Based on this, the GMO Panel considers that the observed increase in NDF in maize MZIR098 does not represent any nutritional concern in animals.

3.4.7. Post-market monitoring of GM food and feed

The GMO Panel concludes that maize MZIR098 does not represent a nutritional concern and is as safe as the conventional counterpart and commercial non-GM maize reference varieties tested. No post-market monitoring (EFSA GMO Panel, 2011a) of food/feed is considered necessary.

3.4.8. Conclusion on the food and feed safety assessment

The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the eCry3.1Ab, mCry3A and PAT proteins as expressed in maize MZIR098, and finds no evidence that the genetic modification would change the overall allergenicity of maize MZIR098. In the context of this application, the consumption of food/feed from maize MZIR098 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that maize MZIR098 is as safe as the conventional counterpart and non-GM maize reference varieties tested, and no post-market monitoring of food/feed is considered necessary.

3.5. Environmental risk assessment and monitoring plan[47]

3.5.1. Environmental risk assessment

Considering the scope of the application EFSA-GMO-DE-2017-142, which excludes cultivation, the environmental risk assessment (ERA) of maize MZIR098 mainly takes into account: (1) the exposure of microorganisms to recombinant DNA in the gastrointestinal tract of animals fed GM material and of microorganisms present in environments exposed to faecal material of these animals (manure and faeces); and (2) the accidental release into the environment of viable maize MZIR098 grains during transportation and/or processing (EFSA GMO Panel, 2010a).

[47] Dossier: Part II – Sections 5, 6 and 7; Additional information: 16/12/2019.

3.5.1.1. Persistence and invasiveness of the GM plant

Maize is highly domesticated, not winter hardy in colder regions of Europe, and generally unable to survive in the environment without appropriate management. Occasional feral GM maize plants may occur outside cultivation areas in the EU (e.g. Pascher, 2016), but survival is limited mainly by a combination of low competitiveness, absence of a dormancy phase and susceptibility to plant pathogens, herbivores and cold climate conditions (OECD, 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 are currently limited and transient.

It is unlikely that the intended traits of event MZIR098 will provide a selective advantage to maize plants, except when they are exposed to glufosinate-ammonium-containing herbicides or infested by coleopteran pests that are susceptible to the eCry3.1Ab and mCry3A proteins. However, this fitness advantage will not allow maize MZIR098 to overcome other biological and abiotic factors (described above) limiting plant's persistence and invasiveness. Therefore, the presence of the intended traits will not affect the persistence and invasiveness of the GM plant.

In conclusion, the GMO Panel considers it unlikely that maize MZIR098 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 MZIR098 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 horizontal gene transfer (HGT) of DNA, or through vertical gene flow via cross-pollination from feral plants originating from spilled grains.

Plant-to-microorganism gene transfer

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, 2009c).

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.

In addition to homology-based recombination processes, at a lower transformation rate, the non-homologous end joining and microhomology-mediated end joining are theoretically possible (Hülter and Wackernagel, 2008; EFSA, 2009c). Independently of the transfer mechanism, the GMO Panel did not identify a selective advantage that a theoretical HGT would provide to bacterial recipients in the environment.

The updated bioinformatic analysis of the event MZIR098 revealed sufficient length and sequence identity for homologous recombination for the three copies of the *nos* terminator with the same, single site in *A. tumefaciens* and *A. rhizogenes*. Because of its limited length (~ 250 bp), no increased potential for facilitated HGT by double homologous recombination was identified.

In summary, there is no indication for an increased likelihood of horizontal transfer of DNA from maize MZIR098 to bacteria. Given the nature of the recombinant DNA, the GMO Panel identified no safety concern linked to an unlikely but theoretically possible HGT.

Plant-to-plant gene transfer

The potential for occasional feral GM maize MZIR098 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, 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, even if exposed to the intended herbicide or infested by coleopteran pests that are susceptible to the eCry3.1Ab and mCry3A proteins.

3.5.1.3. Interactions of the GM plant with target organisms

Taking the scope of the application EFSA-GMO-DE-2017-142 (no cultivation) into account, potential interactions of occasional feral maize MZIR098 plants arising from grain import spills with the target organisms are not considered a relevant issue.

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

Given that environmental exposure of non-target organisms to spilled GM grains or occasional feral GM maize plants arising from spilled maize MZIR098 grains is limited and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions of maize MZIR098 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

Given that environmental exposure to spilled grains or occasional feral maize MZIR098 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

The objectives of a post-market environmental monitoring (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 post-market environmental monitoring plan provided by the applicant (EFSA GMO Panel, 2011b).

As the ERA did not identify potential adverse environmental effects from the maize MZIR098, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for maize MZIR098 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 PMEM report on an annual basis and a final report at the end of the authorisation period.

The GMO Panel considers that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of maize MZIR098. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

In the context of annual PMEM reports, the applicant could further fine-tune future 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 the maize MZIR098 would differ from conventional maize varieties in its ability to persist under European environmental conditions. Considering the scope of the application EFSA-GMO-DE-2017-142, interactions of occasional feral maize MZIR098 plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from maize MZIR098 to bacteria does not indicate a safety concern. Therefore, considering the introduced traits, the outcome of the agronomic and phenotypic analysis, the routes and levels of exposure, the GMO Panel concludes that maize MZIR098 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 MZIR098.

4. Conclusions

The GMO Panel was asked to carry out a scientific assessment of maize MZIR098 for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003.

The molecular characterisation data establish that maize MZIR098 contains a single insert consisting of one copy of the eCry3.1Ab, mCry3A and PAT expression cassettes. Bioinformatic analyses of the sequences encoding the newly expressed proteins and other ORFs present within the insert or spanning the junctions between the insert and genomic DNA does not raise concerns that need additional food/feed safety considerations. The stability of the inserted DNA and of the introduced traits was confirmed over several generations. The methodology used to quantify the levels of the eCry3.1Ab, mCry3A and PAT proteins is considered adequate. The protein characterisation data comparing the structural, biochemical and functional properties of plant- and microbe-produced eCry3.1Ab, mCry3A and PAT proteins indicate that these proteins are equivalent, and the microbe-produced protein can be used in safety studies.

None of the identified differences in the agronomic/phenotypic and compositional characteristics tested between maize MZIR098 and its conventional counterpart needs further assessment, with the exception of NDF in grains. The NDF difference in grains was further assessed for safety and nutritional relevance and raises no concerns.

The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the eCry3.1Ab, mCry3A and PAT proteins as expressed in maize MZIR098, and finds no evidence that the genetic modification would change the overall allergenicity of maize MZIR098. In the context of this application, the consumption of food/feed from maize MZIR098 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that maize MZIR098 is as safe as the conventional counterpart and non-GM maize reference varieties tested, and no post-market monitoring of food/feed is considered necessary.

There is a very low likelihood of environmental effects resulting from the accidental release of viable grains from maize MZIR098 into the environment. The PMEM plan and reporting intervals are in line with the intended uses of maize MZIR098.

Based on the relevant publications retrieved through systematic literature searches, the GMO Panel does not identify any safety issues pertaining to the intended uses of maize MZIR098. In the context of annual PMEM reports, the applicant could further fine-tune future literature searches according to the GMO Panel recommendations.

In conclusion, the GMO Panel considers that maize MZIR098, as described in this application, is as safe as its conventional counterpart and the non-GM maize reference varieties tested with respect to potential effects on human and animal health and the environment.

5. Documentation as provided to EFSA (if appropriate)

- Letter from the Competent Authority of Germany received on 02 May 2017 concerning a request for authorization of the placing on the market maize MZIR098 submitted in accordance with Regulation (EC) No 1829/2003 by Syngenta Crop Protection N.V./S.A.
- Application EFSA-GMO-BE-2016-138 validated by EFSA, 11 August 2017.
- Request for supplementary information to the applicant, 01 September 2017.
- Receipt of supplementary information from the applicant, 27 October 2017.
- Receipt of spontaneous information from the applicant, 27 October 2017.
- Request for supplementary information to the applicant, 13 November 2017.
- Request for supplementary information to the applicant, 04 December 2017.
- Request for supplementary information to the applicant, 15 February 2018.
- Request for supplementary information to the applicant, 08 March 2018.
- Receipt of supplementary information from the applicant, 20 April 2018.
- Receipt of supplementary information from the applicant, 20 June 2018.
- Request for supplementary information to the applicant, 20 July 2018.
- Request for supplementary information to the applicant, 27 July 2018.
- Request for supplementary information to the applicant, 11 October 2018.
- Receipt of supplementary information from the applicant, 29 November 2018.
- Receipt of supplementary information from the applicant, 28 February 2019.
- Receipt of supplementary information from the applicant, 15 May 2019.
- Request for supplementary information to the applicant, 14 June 2019.
- Receipt of supplementary information from the applicant, 16 December 2019.
- Request for supplementary information to the applicant, 23 December 2019.
- Receipt of supplementary information from the applicant, 22 January 2020.
- Request for supplementary information to the applicant, 27 January 2020.
- Request for supplementary information to the applicant, 10 February 2020.
- Receipt of supplementary information from the applicant, 26 February 2020.
- Receipt of spontaneous information from the applicant, 26 February 2020.
- Request for supplementary information to the applicant, 13 March 2020.
- Receipt of supplementary information from the applicant, 30 April 2020.
- Receipt of spontaneous information from the applicant, 30 April 2020.

References

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

Eastham K and Sweet J, 2002. Genetically modified organisms (GMOs): The significance of gene flow through pollen transfer. Environmental Issue Report No 28. European Environment Agency, Copenhagen, Denmark. Available online: http://www.eea.europa.eu/publications/environmental_issue_report_2002_28

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

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

EFSA (European Food Safety Authority), 2009c. Statement of EFSA on the consolidated presentation of the joint Scientific Opinion of the GMO and BIOHAZ Panels on the "Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants" and the Scientific Opinion of the GMO Panel on "Consequences of the Opinion on the Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants on Previous EFSA Assessments of Individual GM Plants". EFSA Journal 2009;8(6):1108, 107 pp. https://doi.org/10.2903/j.efsa.2009.1108

EFSA (European Food Safety Authority), 2010. Application of systematic review methodology to food and feed safety assessments to support decision making. EFSA Journal 2020;8(6):1637, 90 pp. https://doi.org/10.2903/j.efsa.2010.1637

EFSA (European Food Safety Authority), 2014. Explanatory statement for the applicability of the Guidance of the EFSA Scientific Committee on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed for GMO risk assessment. EFSA Journal 2014;12(10):3871, 25 pp. https://doi.org/10.2903/j.efsa.2014.3871

EFSA (European Food Safety Authority), 2015. Use of EFSA Comprehensive European Food Consumption Database for estimating dietary exposure to genetically modified foods. EFSA Journal 2015;13(2):4034, 11 pp. https://doi.org/10.2903/j.efsa.2015.4034

EFSA (European Food Safety Authority), 2016. Relevance of new scientific evidence on the occurrence of teosinte in maize fields in Spain and France for previous environmental risk assessment conclusions and risk management recommendations on the cultivation of maize events MON810, Bt11, 1507 and GA21. EFSA Supporting Publication 2016;EN-1094, 13 pp. https://doi.org/10.2903/j.efsa.2016.EN-1094

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

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010b. Statistical considerations for the safety evaluation of GMOs. EFSA Journal 2010;8(1):1250, 59 pp. https://doi.org/10.2903/j.efsa.2010.1250

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

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2011b. Guidance on the post-market environmental monitoring (PMEM) of genetically modified plants. EFSA Journal 2011;9(8):2316, 40 pp. https://doi.org/10.2903/j.efsa.2011.2316

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2015. Guidance on the agronomic and phenotypic characterisation of genetically modified plants. EFSA Journal 2015;13(6):4128, 44 pp. https://doi.org/10.2903/j.efsa.2015.4128

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Birch AN, Casacuberta J, De Schrijver A, Gralak MA, Guerche P, Jones H, Manachini B, Mess_ean A, Nielsen EE, Nogu_e F, Robaglia C, Rostoks N, Sweet J, Tebbe C, Visioli F, Wal J-M, Paraskevopoulos K and Lanzoni A, 2018a. Statement complementing the EFSA Scientific Opinion on application (EFSA-GMO-DE-2011-95) for the placing on the market of genetically modified maize 5307 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Syngenta Crop Protection AG taking into consideration an additional toxicological study. EFSA Journal 2018a;16(4):5233, 9 pp. https://doi.org/10.2903/j.efsa.2018.5233

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Bresson J-L, Dalmay T, Dewhurst IC, Epstein MM, Firbank LG, Guerche P, Hejatko J, Moreno FJ, Mullins E, Nogue F, Rostoks N, Sanchez Serrano JJ, Savoini G, Veromann E, Veronesi F, Devos Y, Ardizzone M, Neri FM, Papadopoulou N, De Sanctis G, Dumont AF, Gennaro A, Gomez Ruiz JA and Paraskevopoulos K, 2018b. Scientific Opinion on the assessment of genetically modified maize MZHG0JG for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (application EFSA-GMO-DE-2016-133). EFSA Journal 2018b;16(11):5469, 26 pp. https://doi.org/10.2903/j.efsa.2018.5469

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition, and Allergies), 2010. Scientific Opinion on Dietary Reference Values for carbohydrates and dietary fibre. EFSA Journal 2010;8(3):1462, 77 pp. https://doi.org/10.2903/j.efsa.2010.1462

EFSA Scientific Committee, 2011. EFSA guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed. EFSA Journal 2011;9(12):2438, 21 pp. https://doi.org/10.2903/j.efsa.2011.2438

EFSA (European Food Safety Authority), Devos Y, Guajardo IM, Glanville J and Waigmann E, 2017a. Explanatory note on literature searching conducted in the context of GMO applications for (renewed) market authorisation and annual post-market environmental monitoring reports on GMOs authorised in the EU market. EFSA supporting publications 2017;14(4):EN-1207, 48 pp. https://doi.org/10.2903/sp.efsa.2017.en-1207

EFSA (European Food Safety Authority), Gennaro A, Gomes A, Herman L, Nogu_e F, Papadopoulou N and Tebbe C, 2017b. Technical report on the explanatory note on DNA sequence similarity searches in the context of the assessment of horizontal gene transfer from plants to microorganisms. EFSA supporting publications 2017;14(7):EN-1273, 11 pp. https://doi.org/10.2903/sp.efsa.2017.en-1273

EFSA (European Food Safety Authority), Gomez Ruiz JA, Bresson J-L, Frenzel T and Paoletti C, 2019. Statement on the human dietary exposure assessment to newly expressed proteins in GM foods. EFSA Journal 2019;17(7):5802, 18 pp. https://doi.org/10.2903/j.efsa.2019.5802

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

Hülter N and Wackernagel W, 2008. Double illegitimate recombination events integrate DNA segments through two different mechanisms during natural transformation of *Acinetobacter baylyi*. Molecular Microbiology, 67, 984–995.

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

Negrotto D, Jolley M, Beer S, Wenck AR and Hansen G, 2000. The use of phosphomannose-isomerase as a selectable marker to recover transgenic maize plants (Zea mays L.) via Agrobacterium transformation. Plant Cell Reports, 19, 798–803.
OECD (Organisation for Economic Co-operation and Development), 1998. Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents, OECD Publishing, Paris.
OECD (Organisation for Economic Co-operation and Development), 2002. Consensus Document on compositional considerations for new varieties of maize (*Zea mays*): key food and feed nutrients, anti-nutrients and secondary plant metabolites. Series on the Safety of Novel Food and Feeds (ENV/JM/MONO(2002)25), No 6, 1–42.
OECD (Organisation for Economic Co-operation and Development), 2003. Consensus Document on the biology of *Zea mays* subsp. Mays (Maize). Series on Harmonisation of Regulatory Oversight in Biotechnology (ENV/JM/MONO(2003)11), No 27, 1–49.
OECD (Organisation for Economic Co-operation and Development), 2013. Guidance document on residues in livestock. In: Series on Pesticides No 73. ENV/JM/MONO(2013)8, 4 September 2013.
Palaudelmàs M, Peñas G, Melé E, Serra J, Salvia J, Pla M, Nadal A and Messeguer J, 2009. Effect of volunteers on maize gene flow. Transgenic Research, 18, 583–594.
Pascher K, 2016. Spread of volunteer and feral maize plants in Central Europe: recent data from Austria. Environmental Sciences Europe, 28, 30. https://doi.org/10.1186/s12302-016-0098-1
Polevoda B and Sherman F, 2000. Na-terminal acetylation of eukaryotic proteins. Journal of Biological Chemistry, 275, 36479–36482.
Steinberg P, van der Voet H, Goedhart PW, Kleter G, Kok EJ, Pla M, Nadal A, Zeljenkovà D, Aláčová R, Babincová J, Rollerová E, Jad'ud'ová S, Kebis A, Szabova E, Tulinská J, Líšková A, Takácsová M, Lehotská Mikušová M, Krivošíková Z, Spök A, Racovita M, de Vriend H, Alison R, Alison C, Baumgärtner W, Becker K, Lempp C, Schmicke M, Schrenk D, Pöting A, Schiemann J and Wilhelm R, 2019. Lack of adverse effects in subchronic and chronic toxicity/carcinogenicity studies on the glyphosate-resistant genetically modified maize NK603 in Wistar Han RCC rats. Archives of Toxicology, 93, 1095. https://doi.org/10.1007/s00204-019-02400-1
Sys C, Van Ranst E, Debaveye J and Beernaert F, 1993. Land Evaluation. Part III: Crop requirements. Agricultural Publication No. 7. — Brussels, General Administration for Development Cooperation, 199 pp.
Trtikova M, Lohn A, Binimelis R, Chapela I, Oehen B, Zemp N, Widmer A and Hilbeck A, 2017. Teosinte in Europe – searching for the origin of a novel weed. Scientific Reports, 7, 1560. https://doi.org/10.1038/s41598-017-01478-w
Windels P, Alcalde E, Lecoq E, Legris G, Pleysier A, Tinland B and Wandelt C, 2008. General surveillance for import and processing: the EuropaBio approach. Journal of Consumer Protection and Food Safety, 3, 14–16.

Abbreviations

bw	Body weight
dw	Dry weight
ELISA	Enzyme-linked immunosorbent assay
fw	Fresh weight
GLP	Good laboratory practice
GMO	Genetically modified organism
HGT	Horizontal gene transfer
LOD	Limit of detection
LOQ	Limit of quantification
NDF	Neutral detergent fibre
NGS	Next-generation sequencing
ORF	Open reading frames
PMEM	Post-market environmental monitoring

Appendix A – List of additional unpublished studies performed by or on behalf of the applicant with regard to the evaluation of the safety of the food and feed for humans, animal and the environment for maize MZIR098

Study identification	Title
9047-100671	Cytotoxicity testing of the eCry3.1Ab protein as contained in test substance ECRY3.1AB-0208
33805	eCry3.1Ab – A 28 day repeat dose toxicity study by oral gavage in rats
C1413	Quantitation of endogenous lipid transfer protein (LTP) allergen in MZIR098 maize using mass spectrometry
SSB-014-08	Bioactivity of test substance ECRY3.1AB-0208
SSB-024-08	*In vitro* digestibility of eCry3.1Ab protein as contained in test substance ECRY3.1AB-0208 and in 5307 maize under simulated mammalian gastric conditions
SSB-234-10	Effect of buffer ionic strength on the *in vivo* digestibility of eCry3.1Ab protein under simulated mammalian gastric conditions
TK0117440 A1	Collection of agronomic data and forage and grain samples for compositional analysis of maize events MZHG0JG and MZIR098 grown in the USA in 2013
TK0117449 A2	Agronomic performance of MZIR098 maize grown in the USA in 2013
TK0117452 A1	Compositional analysis of forage and grain from MZIR098 grown during 2013 in the USA
TK0117455	Agronomic and phenotypic assessment of transgenic maize event MZIR098 grown during 2013-2014 in Argentina
TK0117467 A1	Pollen viability and morphology of Event MZIR098 maize
TK0117498 Vol 1&2	Event MZIR098 maize – Determination of the chromosomal location of the transgenic locus
TK0204931	Storage stability assessment of microbially produced test substance MCRY3A-0102 containing modified Cry3A protein
TK0214830	Storage stability assessment of microbially produced test substance MCRY3A-0108 containing mCry3A protein
TK0219348 A3	Storage stability assessment of microbially produced test substance PAT-0109 containing phosphinothricin acetyltransferase (PAT) protein
TK0220453	Peptide mass coverage analysis of phosphinothricin acetyltransferase (PAT) protein produced in recombinant *Escherichia coli*
TK0256254	Confirmation of absence of *Agrobacterium tumefaciens* in the MZIR098 F1 generation
TK0285530	*In vitro* digestibility of eCry3.1Ab protein under simulated mammalian gastric conditions for China
TK0326215	Effect of temperature on the stability of eCry3.1Ab protein
TK0326289	Effect of temperature on the stability of phosphinothricin acetyltransferase (PAT) protein
WIL-639226	A single-dose oral gavage toxicity study of ECRY3.1AB-0110 in CD-1 mice with a 14-day recovery period
38073	eCry3.1Ab- A 28 Day Repeat Dose Toxicity Study by Oral Gavage in Mice
WIL-639031	ECRY3.1AB-0208: Single-Dose Oral (Gavage) Toxicity Study in Mice with a 14-Day Observation Period
WIL-639181	PAT: Acute Oral (Gavage) Toxicity Study in Mice with a 14-Day Observation Period
AM7301	Acute oral toxicity study of modified Cry3A Protein (MCRY3A-0102) in the mouse

Appendix B – List of relevant publications identified by the applicant through systematic literature searches (January 2007–March 2020)

References
Nagy A, Pauk J, Takacs K and Gelencser E, 2008. Nutritional evaluation of the proteins of broad range herbicide resistant spring wheat (*Triticum aestivum* L.) lines II. Resistance to digestion of marker proteins in rat model. Acta Alimentaria, 37, 159–166.
Schafer BW, Embrey SK and Herman RA, 2016. Rapid simulated gastric fluid digestion of in seed/grain proteins expressed in genetically engineered crops. Regulatory Toxicology and Pharmacology, 81, 106–112.
Fard NA, Minuchehr Z, Mousavi A. 2013. In Silico Allergenicity Assessment of Novel Proteins Derived from GMHR Crops. In Ortuno, F.R.I. (ed), Proceedings IWBBIO 2013: International Work-Conference on Bioinformatics and Biomedical Engineering. Copicentro Granada S L, Granada, Spain, 275.
Siruguri V, Bharatraj DK, Vankudavath RN, Mendu VV, Gupta V, Goodman RE. 2015. Evaluation of Bar, Barnase, and Barstar recombinant proteins expressed in genetically engineered Brassica juncea (Indian mustard) for potential risks of food allergy using bioinformatics and literature searches. [Erratum appears in Food Chem Toxicol. 2015 Dec;86:386–7]. Food & Chemical Toxicology, 83, 93–102.
Sun HJ, Kang HG, Bae TW, Cho TG, Kim J, Lim PO, Riu KZ, Lee HY. 2010. Assessment of Phosphinothricin Acetyltransferase (PAT) Degradation From Transgenic Zoysiagrass Digested with Simulated Gastric Fluid (SGF). Journal of Plant Biology, 53, 2, 113–120.
Chen E and Defontes C, 2012. Modified Cry3A toxins and nucleic acid sequences coding therefor. Official Gazette of the United States Patent & Trademark Office Patents.
Chen E and Stacy C, 2007a. Modified Cry3A toxins and nucleic acid sequences coding therefor. Official Gazette of the United States Patent & Trademark Office Patents, JUN 12.
Chen E and Stacy C, 2007b. Modified Cry3A toxins. Official Gazette of the United States Patent & Trademark Office Patents, OCT 2.
Chen E and Stacy C, 2009. Modified Cry3A toxins and nucleic acid sequences coding therefor. Official Gazette of the United States Patent & Trademark Office Patents., AUG 4.
Chen E and Stacy C, 2011. Modified Cry3A toxins and nucleic acid sequences coding therefor. Official Gazette of the United States Patent & Trademark Office Patents., AUG 30.
Chen E and Stacy C, 2012. Modified Cry3A toxins and nucleic acid sequences coding therefor Patent. Official Gazette of the United States Patent & Trademark Office Patents., JUL 10.
Chen E and Defontes C, 2014. Transgenic plants expressing modified CRY3A. Official Gazette of the United States Patent & Trademark Office Patents., JUN 24.
Christ B, Hochstrasser R, Guyer L, Francisco R, Aubry S, Hortensteiner S and Weng J-K, 2017. Non-specific activities of the major herbicide-resistance gene BAR. Nature Plants, 3, 12, 937–945.
Haegele JW and Peterson PA, 2007. The flow of maize pollen in a designed field plot. Maydica, 52, 2, 117–125.
Walters FS, Young S and Graser G, 2020. Meeting technical challenges for protein characterization and surrogate equivalence studies that resulted from insecticidal protein co-expression in maize event MZIR098. Transgenic Research, 29, 109–124.
Hart et al., 2019. Insecticidal proteins. Official Gazette of the United States Patent & Trademark Office Patents, March 26.