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SCIENTIFIC OPINION

Assessment of genetically modified maize DP51291 (application GMFF-2021-0071)

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Abstract

Genetically modified maize DP51291 was developed to confer control against susceptible corn rootworm pests and tolerance to glufosinate-containing herbicide; these properties were achieved by introducing the *ipd072Aa, pmi* and *mo-pat* expression cassettes. 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 DP51291 and its conventional counterpart needs further assessment, except for phosphorus in forage and manganese, proline, oleic acid (C18:1) and linoleic acid (C18:2) in grain, which do not raise safety and nutritional concerns. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the IPD072Aa, PAT and PMI proteins as expressed in maize DP51291 and finds no evidence that the genetic modification would change the overall allergenicity of maize DP51291. In the context of this application, the consumption of food and feed from maize DP51291 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that maize DP51291 is as safe as its conventional counterpart and non-GM maize varieties tested, and no post-market monitoring of food/feed is considered necessary. In the case of accidental release of viable maize DP51291 grains into the environment, this would not raise environmental safety concerns. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of maize DP51291. The GMO Panel concludes that maize DP51291 is as safe as its conventional counterpart and the tested non-GM maize varieties with respect to potential effects on human and animal health and the environment.

KEYWORDS

DP51291, genetic engineering, GM, import and processing, IPD072Aa, maize (*Zea mays*), PAT, PMI

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SUMMARY

Following the submission of application GMFF-2021-0071 under Regulation (EC) No 1829/2003 from Corteva Agriscience LLC (referred to hereafter as 'the applicant'), the Panel on Genetically Modified Organisms of the European Food Safety Authority (referred to hereafter as 'GMO Panel') was asked to deliver a Scientific Opinion on the safety of genetically modified (GM) herbicide tolerant and insect resistant maize (*Zea mays*) DP51291 according to Regulation (EU) No 503/2013. The scope of application GMFF-2021-0071 is for import, processing, and food and feed uses within the European Union (EU) of maize DP51291 and does not include cultivation in the EU.

In this Scientific Opinion, the GMO Panel reports on the outcome of its risk assessment of maize DP51291 according to the scope of the application GMFF-2021-0071. The GMO Panel conducted the assessment of maize DP51291 in line with the principles described in Regulation (EU) No 503/2013 and its applicable guidelines for the risk assessment of GM plants. The molecular characterisation data establish that maize DP51291 contains a single insert consisting of one copy of the *ipd072Aa, pmi* and *mo-pat* expression cassettes. Bioinformatics 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, do not raise any safety concerns. The stability of the inserted DNA and of the introduced trait is confirmed over several generations. The methodology used to quantify the levels of the IPD072Aa, PAT and PMI proteins is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of plant and previously assessed microbe-produced IPD072Aa indicate that these proteins are equivalent.

Considering the selection of test materials, the field trial sites and the associated management practices and the agronomic-phenotypic characterisation as an indicator of the overall field trial quality, the GMO Panel concludes that the field trials are appropriate to support the comparative analysis. None of the identified differences in the agronomic/phenotypic and compositional characteristics tested between maize DP51291 and its conventional counterpart needs further assessment, except for phosphorus in forage and manganese, proline, oleic acid (C18:1) and linoleic acid (C18:2) in grain, which does not raise safety and nutritional concerns. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the IPD072Aa, PAT and PMI proteins as expressed in maize DP51291 and finds no evidence that the genetic modification would change the overall allergenicity of maize DP51291. In the context of this application, the consumption of food and feed from maize DP51291 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that maize DP51291 is as safe as its conventional counterpart and non-GM maize 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 DP51291 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 DP51291.

The GMO Panel considered the overall quality of the performed literature searches acceptable.

Based on the relevant publications identified through the literature searches, the GMO Panel does not identify any safety issue pertaining to the intended uses of maize DP51291.

The GMO Panel concludes that maize DP51291 is as safe as its conventional counterpart and the tested non-GM maize reference varieties with respect to potential effects on human and animal health and the environment.

1 | INTRODUCTION

The scope of the application GMFF-2021-0071 is for food and feed uses, import and processing of maize DP51291 and does not include cultivation in the European Union (EU). Maize DP51291 was developed to confer control against susceptible corn rootworm pests and tolerance to glufosinate-containing herbicide.

1.1 | Background

On 30 January 2023, the European Food Safety Authority (EFSA) received from the Competent Authority of The Netherlands application GMFF-2021-0071 for authorisation of maize DP51291 (Unique Identifier DP-Ø51291-2), submitted by Corteva Agriscience LLC (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003.[1] Following receipt of application GMFF-2021-0071, EFSA informed EU Member States (MS) and the European Commission (EC) 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 Regulation (EC) No 1829/2003 and Regulation (EU) No 503/2013,[3] with the EFSA guidance documents, and, when needed, asked the applicant to supplement the initial application. On 04 May 2023, EFSA declared the application valid.

From validity date, EFSA and the Panel on Genetically Modified Organisms of the European Food Safety Authority (referred to hereafter as 'GMO Panel') endeavoured to respect a time limit of 6 months to issue a scientific opinion on application GMFF-2021-0071. Such time limit was extended whenever EFSA and/or 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 5, 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.[4] The EU Member States had 3 months to make their opinion known on application GMFF-2021-0071 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 DP51291 in the context of its scope as defined in application GMFF-2021-0071.

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.

In addition to the present scientific opinion, EFSA was also asked to report on the particulars listed under Articles 6(5) and 18(5) of Regulation (EC) No 1829/2003, but not to give an opinion on them because they pertain to risk management.

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant submitted a confidential and a non-confidential version of the dossier GMFF-2021-0071 following the EFSA requirements as detailed by EFSA (2021a, 2021b).

In accordance with Art. 38 of the Regulation (EC) No 178/2002[5] and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation, the non-confidential version of the dossier was published in OpenEFSA.[6]

According to Art. 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations,[7] EFSA carried out a public consultation on the non-confidential version of the dossier from 5 May 2023 to 5 August 2023 for which no comments were received.

[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, pp. 1–23.

[2]Available online: https://open.efsa.europa.eu/questions/EFSA-Q-2023-00051.

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

[4]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, pp. 1–38.

[5]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, pp. 1–48.

[6]https://open.efsa.europa.eu/questions/EFSA-Q-2023-00051.

[7]Decision available at: https://www.efsa.europa.eu/sites/default/files/corporate_publications/files/210111-PAs-pre-submission-phase-and-public-consultations.pdf.

The GMO Panel based its scientific assessment of maize DP51291 on the valid application GMFF-2021-0071, additional information provided by the applicant during the risk assessment, relevant scientific comments submitted by EU MS and relevant peer-reviewed scientific publications. As part of this comprehensive information package, the GMO Panel received additional 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.

2.2 | Methodologies

The GMO Panel conducted its assessment in line with the principles described in Regulation (EU) No 1829/2003, the applicable guidelines (i.e. EFSA GMO Panel, 2010a, 2011a, 2011b, 2015; EFSA Scientific Committee, 2011) and explanatory notes and statements (i.e. EFSA, 2010, 2014, 2017, 2018, 2019a, 2019b; EFSA GMO Panel, 2010b, 2018) for the risk assessment of GM plants.

For this application, in the context of the contracts [OC/EFSA/GMO/2018/04 literature search], [OC/EFSA/GMO/2020/01 sequencing quality check], [OC/EFSA/GMO/2021/06 BI] and [EOI/EFSA/2022/01 – CT NIF 2023 02 TOX] the contractors performed preparatory work for the evaluation of the applicant's literature search, the completeness and quality of DNA sequencing information, the bioinformatic analyses on maize DP51291 and methods applied for the statistical analysis of the 90-day toxicity study.

3 | ASSESSMENT

3.1 | Introduction

Maize DP51291 expresses the IPD072Aa protein for control of susceptible corn and rootworm pests, phosphinothricin acetyltransferase (PAT) protein that confers tolerance to the glufosinate herbicide and the phosphomannose isomerase (PMI) protein that was used as a selectable marker during transformation.

The assessment of herbicide residues relevant for this application is in the remit of the EFSA Plant Health and Pesticides Residues Unit (EFSA, 2015).

3.2 | Systematic literature review[8]

The GMO Panel assessed the applicant's literature searches on maize DP51291, which include a scoping review, according to the guidelines given in EFSA (2010, 2019b).

A systematic review as referred to in Regulation (EU) No 503/2013 has not been provided in support to the risk assessment of application GMFF-2021-0071. Based on the outcome of the scoping review, the GMO Panel agrees that there is limited value of undertaking a systematic review for maize DP51291 at present.

The GMO Panel considered the overall quality of the performed literature searches acceptable. The literature searches identified eight relevant peer-reviewed publications on maize DP51291 (Appendix B).

None of the relevant publications identified through the literature searches reported information pointing to safety issues associated with maize DP51291 relevant to the scope of this application.

3.3 | Molecular characterisation[9]

3.3.1 | Transformation process and vector constructs

Maize DP51291 was developed using three sequential transformation steps, the first two to introduce a 'landing pad' suitable for the insertion of the intended expression cassette, which was introduced in a third step:

1. *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*)-mediated transformation to insert what could be considered the 'pre-landing pad' sequence. Explants of maize were co-cultured with a disarmed *A. tumefaciens* strain LBA4404 containing the vector PHP50742.
2. Microprojectile co-bombardment of five different plasmids (PHP46438, PHP5096, PHP16072, PHP21139 and PHP31729) to modify the inserted sequence and obtain a final 'landing pad' and to transiently improve plant regeneration to facilitate the transformation process.

[8]Dossier: Part II- Section 7; additional information: 21/11/2023, 24/7/2024.
[9]Dossier: Part II – Section 1.2; additional information: 31/5/2023, 10/8/2023, 10/4/2024 and 8/5/2024.

3. *A. tumefaciens* (also known as *R. radiobacter*)-mediated transformation to insert the intended expression cassettes into the landing pad in the maize genome. Explants of maize were co-cultured with a disarmed *A. tumefaciens* strain AGL1 containing the vector PHP74638.

The first transformation step by *A. tumefaciens* inserts the T-DNA of plasmid PHP50742, which contains the pre-landing pad sequence.

The T-DNA of plasmid PHP50742 contains three expression cassettes, of which one cassette (*ip3-h5*) is flanked by *loxP* sites, recognised by a Cre recombinase in the subsequent step; and two cassettes (*pmi* and *mo-pat*) are flanked by FRT1 and FRT87 recombination sites, intended to facilitate a flippase-mediated recombination after the second transformation step.

The cassette flanked by *loxP* sites consists of the following genetic elements:

– The *ip3-h5* gene cassette consists of the duplicated promoter sequence from the citrus yellow mosaic virus (dCYMV) with the intron 1 of the alcohol dehydrogenase gene from *Z. mays*; the coding sequence of the modified *cry3Aa* gene from *Bacillus thuringiensis* (*ip3-h5*); the nos terminator from *A. tumefaciens*.

The two cassettes flanked by FRT1 and FRT87 recombination sites consist of the following genetic elements:

– The *pmi* gene cassette consists of the *pmi* coding sequence of the *phosphomannose isomerase* (*pmi*) gene from *Escherichia coli*, and the terminator region from the *pinII* gene of *Solanum tuberosum*, followed by an additional terminator region from *Z19* gene of *Z. mays*. The expression of the *pmi* gene is driven by the promoter region from the maize ubiquitin *gene 1* of *Z. mays* (*ubiZM1*), including the 5′ untranslated region (5′ UTR) and intron, located upstream of the FRT1 site and not involved in the flippase recombination of the subsequent step.
– The *mo-pat* gene cassette consists of the promoter and intron region of the *os-actin* gene, the maize-optimised version of the *pat* coding sequence of the phosphinothricin acetyltransferase gene (*pat*) from *Streptomyces viridochromogenes* and the CaMV 35S terminator.

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

In the second transformation step a microprojectile co-bombardment delivers five plasmids intended to obtain the landing pad (PHP46438, PHP5096, PHP16072) and to improve transiently plant regeneration (PHP21139, PHP31729). The Cre recombinase carried by plasmid PHP16072 removes the *ip3-h5* gene, leaving one *loxP* site in the landing pad. The flippase recombinase expressed by plasmid PHP5096 removes the *pmi* and *mo-pat* cassettes, substituting them with the *nptII* and *AmCyan1* cassettes, placed between FRT1 and FRT87 recombination sites. The WUS2 and ODP2 proteins expressed by PHP21139 and PHP31729, respectively, enhance the plant regeneration process. The *cre* recombinase, the flippase, the *wus2* and *odp2* genes are transiently expressed and they are not integrated into the DP51291 maize genome.

The plasmid PHP46438, used to insert the *nptII* and *AmCyan1* genes, contains two cassettes, consisting of the following genetic elements, flanked by FRT1 and FRT87 recombination sites:

– The *nptII* cassette consists of the coding sequence of the *nptII* gene from *E. coli* and the terminator region from the *pinII* gene of *S. tuberosum*.

– *The Amcyan1* cassette consists of the promoter region from the maize ubiquitin gene 1 of *Z. mays* (ubiZM1) including the 5′ untranslated region (5′ UTR) and intron, the coding region of the modified cyan fluorescent protein from *Anemonia majano*, the terminator region from the *pinII* gene of *S. tuberosum*.

The plasmid PHP5096, used to deliver the flippase recombinase, contains one cassette consisting of the following genetic elements:

– The *mo-Flp* gene cassette consists of the promoter region from the maize ubiquitin gene 1 of *Z. mays* (*ubiZM1*) including the 5′ untranslated region (5′ UTR) and intron, the coding region of maize-optimised flippase gene (*mo-Flp*) of *Saccharomyces cerevisiae* and the terminator region from the *pinII* gene of *S. tuberosum*.

The plasmid PHP16072, used to express the *cre* recombinase, contains one cassette consisting of the following genetic elements:

– The *mo-cre* gene cassette consists of the promoter region from the maize ubiquitin gene 1 of *Z. mays* (*ubiZM1*) including the 5′ untranslated region (5′ UTR) and intron, the maize-optimised exon 1 and exon 2 of the *cre* recombinase gene (*mo-cre*) from bacteriophage P1 of *E. coli*, separated by an intron region from the *LS1* (*st-LS1*) gene of *S. tuberosum* and the terminator region from the *pinII* gene of *S. tuberosum*.

The plasmid PHP21139, used to express the Wuschel *2* protein to improve plant regeneration, contains one expression cassette consisting of the following genetic elements:

– The *zm-wus2* cassette consists of the promoter region the maize *In2-2* gene, the coding sequence of the *wuschel2* (*wus2*) gene of *Z. mays*, including the 5′ and 3′ UTRs and two introns, and the terminator region from the *In2-1* gene of *Zea mays*.

The plasmid PHP31729, used to express the ODP2 protein to improve plant regeneration, contains one expression cassette consisting of the following genetic elements:

– The *zm-odp2* gene cassette consists of the promoter region from the maize oleosin gene, the coding sequence of the ovule development protein 2 (*odp2*) gene of *Z. mays* and the terminator region from the nopaline synthase gene of *A. tumefaciens*.

The backbones of all the above-mentioned plasmids contain elements necessary for the maintenance and selection of the plasmids in bacteria.

In the third step an *Agrobacterium*-mediated transformation was used to deliver plasmid PHP74638. The T-DNA of PHP74638 contains seven expression cassettes in total, three of which flanked by the FRT1 and FRT87 sites. Only the region contained between the FRT1 and FRT87 sites is integrated through a flippase recombination event, replacing the *nptII* and *AmCyan1* cassettes introduced in the landing pad in the previous step.

The four expression cassettes outside of the FRT1 and FRT87 sites (*zm-wus2*, *zm-odp2*, *mo-Flp* and *DsRed2*), which are transiently expressed, and are not integrated into the DP51291 maize genome, consist of the following genetic elements:

– The *zm-wus2* cassette consists of the promoter region of the nopaline synthase gene of *A. tumefaciens*, the *wus2* coding sequence with 5′ and 3′ UTRs of the Wuschel2 (*wus2*) gene of *Z. mays* and the terminator region from the *pin*II gene of *S. tuberosum*.
– The *zm-odp2* gene cassette consists of the promoter region from the maize ubiquitin gene 1 of *Z. mays* (*ubiZM1*) including the 5′ untranslated region (5′ UTR) and intron, the coding sequence of the ovule development protein 2 (*odp2*) gene of *Z. mays* and the terminator region from the pinII gene of *S. tuberosum*. An additional terminator is present between the second and third cassettes: the terminator region from the 19-kDa zein (*Z19*) gene of *Z. mays*.
– The *mo-Flp* gene cassette consists of the promoter region from the maize ubiquitin gene 1 of *Z. mays* (*ubiZM1*) including the 5′ untranslated region (5′ UTR) and intron, maize-optimised exon 1 and exon 2 of the flippase (*Flp*) gene of *S. cerevisiae*, separated by an intron region from the LS1 (st-LS1) gene of *S. tuberosum* and the terminator region from the *pinII* gene of *S. tuberosum*.
– The *DsRed2* gene cassette consists of the 35S enhancer region from the cauliflower mosaic virus genome (CaMV 35S enhancer), the promoter region of the lipid transfer protein gene (*Ltp2*) from *Hordeum vulgare*, a modified coding sequence of the red fluorescent gene (DsRed2) from *Discosoma* sp. and the 35S terminator region from the cauliflower mosaic virus genome (CaMV 35S terminator). An additional copy of the CaMV 35S terminator is intended to prevent transcriptional interference between cassettes.

The three expression cassettes between FRT1 and FRT87 sites (*pmi*, *mo-pat* and *ipd072Aa*) that are integrated into the maize genome consist of the following genetic elements:

– The *pmi* expression cassette in PHP74638 lacks the promoter. However, following the integration in the 'landing pad', the *pmi* expression is driven by maize (*Z. mays*) ubiquitin promoter *ubiZM1*, 5′-UTR and intron, provided by the 'landing pad'. In PHP74638, the phosphomannose isomerase (*pmi*) gene contains the coding sequence from *E. coli*, including its 5′ and 3′ UTRs, and the terminator from potato (*S. tuberosum*) proteinase inhibitor II gene (*pinII*). An additional terminator from the maize 19-kDa zein gene (*Z19*) is present to prevent transcriptional interference between cassettes.
– The *mo-pat* expression cassette contains the promoter and intron region of the rice (*Oryza sativa*) actin gene (*os-actin*), the maize codon-optimised coding sequence of the phosphinothricin acetyltransferase gene from *Streptomyces viridochromogenes* (*mo-pat*) and the 35S terminator region from the cauliflower mosaic virus genome (CaMV 35S terminator). Two additional terminators from the sorghum (*Sorghum bicolor*) ubiquitin gene (*sb-ubi*) and from the γ-kafirin gene (*sb-gkaf*) are present to prevent transcriptional interference between cassettes.
– The *ipd072Aa* expression cassette contains the promoter region from the banana streak virus Acuminata Yunnan strain [BSV (AY)], the intron from the maize ortholog of a rice (*O. sativa*) hypothetical protein (zm-HPLV9), the plant codon-optimised coding sequence of the insecticidal protein gene (*ipd072Aa*) from *Pseudomonas chlororaphis* and the terminator region from the *Arabidopsis thaliana at-T9* gene.

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

3.3.2 | Transgene constructs in the GM plant

Molecular characterisation of maize DP51291 was performed by Southern-by-sequencing (SbS) and junction sequence analysis (JSA) to determine insert copy number and to confirm the absence of plasmid backbone sequences and by targeted sequencing on PCR amplified fragments to determine size and organisation of the inserted sequences.

The EFSA GMO Panel assessed the sequencing data and found it compliant with the requirements listed in the EFSA Technical Note (2018), both in terms of the approach, of the coverage and sensitivity. SbS and JSA analyses indicated that maize DP51291 contains a single insert, consisting of a single copy of the DNA regions deriving from PHP50742 and PHP74638. SBS also confirmed the absence of vector backbone sequences and the absence of sequences deriving from all other plasmids used in the transformation steps and not meant to be integrated in the maize DP51291 genome.

Sanger sequencing of PCR amplified fragments determined the nucleotide sequence of the entire maize DP51291 insert consisting of 12,203 bp nucleotide sequence together with 1032 bp of the 5′ and 1158 bp of the 3′ flanking regions. The Sanger analysis revealed that the insert in maize DP51291 is identical to the intended landing pad sequence from plasmid PHP50742 and the DNA sequence from plasmid PHP74638. 3 bp of the right border region and 12 bp of the left border region from plasmid PHP50742 were incorporated into the genome.

A comparison with the pre-insertion locus indicated that 113 bp were deleted from the maize genomic DNA.

The possible interruption of known endogenous maize genes by the insertion in maize DP51291 was evaluated by bioinformatic analyses of the pre-insertion locus and of the genomic sequences flanking the insert. The results of these analyses do not indicate the interruption of any known endogenous gene in maize DP51291.

The results of segregation (see Section 3.3.5) are compatible with a single insertion in the nuclear genome, in agreement with the conclusions of the bioinformatic analyses.

Bioinformatic analyses of the amino acid sequence of the newly expressed proteins reveal no significant similarities to toxins and allergens for IPD072Aa and PAT. An eight amino acids perfect match to a putative alpha-parvalbumin from *Rana* species was found for PMI. This was previously assessed and found not to raise safety concerns (EFSA GMO Panel, 2012). In addition, bioinformatic analyses of the newly created open reading frames (ORFs) within the insert and spanning the junctions between the insert and genomic DNA, indicated that five ORFs (DP51291_235, DP51291_341, DP51291_562, DP51291_563 and DP51291_723) exceeded the allergenicity assessment threshold of 35% identity using an 80 amino acid sliding window approach. ORF DP51291_235 is in the same orientation but in a different reading frame to the PAT ORF and does not contain any in-frame translational start codons (ATG). ORF DP51291_341 is in the same orientation but in a different reading frame to the IPD072Aa ORF and does not contain any in-frame translational start codons (ATG). ORFs DP51291_562, DP51291_563 and DP51291_723 are in the reverse orientation and there is no known promoter in their immediate upstream regions. Two short ORFs (DP51291_409 and DP51291_562) contain an eight amino acid perfect match to allergens. However, these ORFs are in the reverse orientation and there is no known promoter in their immediate upstream regions. No significant similarities with toxins were identified for any ORF within the insert and spanning the junctions between the insert and genomic DNA. In conclusion, these analyses indicated that the expression of any ORF showing significant similarities to toxins or allergens in maize DP51291 is unlikely.

In order to assess the possibility for horizontal gene transfer (HGT) by homologous recombination (HR), the applicant performed a sequence identity analysis for maize DP51291 to microbial DNA. The likelihood and potential consequences of plant -to -bacteria gene transfer are described in Section 3.6.1.2.

3.3.3 | Protein characterisation and equivalence

Maize DP51291 expresses three new proteins: IPD072Aa, PAT and PMI. Given the technical restraints in producing large enough quantities from plants, IPD072Aa was recombinantly produced in *E. coli*. A set of biochemical methods was employed to demonstrate the equivalence between the maize DP51291 and *E. coli*-produced IPD072Aa.

IPD072Aa protein characterisation and equivalence

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis showed that both plant and a previously assessed microbe-produced IPD072Aa proteins had the expected molecular weight of ~10 kDa and were comparably immunoreactive to IPD072Aa protein-specific antibodies (EFSA GMO Panel, 2024). Glycosylation detection analysis demonstrated that none of the IPD072Aa proteins was glycosylated. Amino acid sequence analysis of the plant-derived IPD072Aa protein by mass spectrometry (MS) methods showed that the protein matched the deduced sequence as defined by the *ipd072Aa* gene. The sequence analysis data were consistent with the previously analysed microbe-produced IPD072Aa. In addition, the amino acid sequence analysis data showed that the *N*-terminal methionine of the plant-produced IPD072Aa was truncated and one His residue from the His tag used to purify the *E. coli*-produced IPD072Aa remained after cleaving by trypsin treatment. Modifications such as *N*-terminal methionine truncation are common in eukaryotic proteins (Polevoda & Sherman, 2000) and have been previously assessed by the GMO Panel for newly expressed proteins (EFSA GMO Panel, 2017). Functional equivalence was demonstrated by an in vitro assay which showed that plant and the previously assessed microbe-derived IPD072Aa proteins had comparable functional activity.

In conclusion, the protein characterisation data comparing the biochemical, structural and functional properties of plant and previously assessed microbe-produced IPD072Aa indicate that these proteins are equivalent (EFSA GMO Panel, 2024).

PAT protein characterisation

SDS-PAGE and western blot analysis showed that plant -produced PAT protein had the expected molecular weight of ~21 kDa and was immunoreactive to PAT protein-specific antibodies. Glycosylation detection analysis demonstrated that the plant-produced PAT protein was not glycosylated. Amino acid sequence analysis of the plant-derived PAT protein by MS methods showed that the protein matched the deduced sequence as defined by the *pat* gene. In addition, the amino acid sequence analysis data showed that the *N*-terminal methionine of the plant-produced PAT protein was truncated. Such modifications are common in eukaryotic proteins (e.g. Polevoda & Sherman, 2000), and have been previously assessed by the GMO Panel for newly expressed proteins (EFSA GMO Panel, 2017).

PMI protein characterisation

SDS-PAGE and western blot analysis showed that plant-produced PMI protein had the expected molecular weight of ~43 kDa and was immunoreactive to PMI protein-specific antibodies. Glycosylation detection analysis demonstrated that the plant-produced PMI protein was not glycosylated. Amino acid sequence analysis of the plant-derived PMI protein by MS methods showed that the protein matched the deduced sequence as defined by the *pmi* gene. In addition, the MS data showed that the *N*-terminal methionine of the plant-produced PMI protein was acetylated. Such modifications are common in eukaryotic proteins (e.g. Polevoda & Sherman, 2000).

3.3.4 | Information on the expression of the insert

Protein levels of IPD072Aa, PAT and PMI were analysed by an enzyme-linked immunosorbent assay (ELISA) in material harvested in a field trial across six locations in the United States and Canada during the 2021 growing season. Samples analysed included root (BBCH 16, BBCH 19, BBCH 63–65 and BBCH 85), leaf (BBCH 19, BBCH 63–65 and BBCH 85), pollen (BBCH 63–65), forage (BBCH 85) and grain (BBCH 87–99) from plants treated and not treated with glufosinate. The mean values and standard deviations of protein expression levels in grains ($n = 24$), forage ($n = 24$) and pollen (n = 24) of the IPD072Aa, PAT and PMI proteins used to estimate human and animal dietary exposure (see Section 3.5.5) are reported in Table 1.

TABLE 1 Mean values ($n = 24$) and standard deviation of newly expressed protein in grains [μg/g dry weight (dw) and μg/g fresh weight (fw)] pollen and forage (μg/g dw) from maize DP51291.

	Glufosinate treatment			
	Not treated		Treated	
Tissue	μg/g dry weight (dw)	μg/g fresh weight (fw)	μg/g dry weight (dw)	μg/g fresh weight (fw)
Grains (BBCH 87-99)				
IPD072Aa	4.1[a] ± 3.6[b] (0.051–12)[c]	3.4 ± 3.0 (0.043–10)	3.8 ± 3.2 (0.24–11)	3.2 ± 2.7 (0.2–9.2)
PAT	8.3 ± 2.6 (3.3–13)	7.0 ± 2.2 (2.8–11)	8.4 ± 2.5 (4.8–16)	7.1 ± 2.1 (4.0–13)
PMI	7.9 ± 3.3 (3.3–18)	6.6 ± 2.8 (2.8–15)	7.1 ± 1.8 (3.8–11)	6.0 ± 1.5 (3.2–9.2)
Forage (BBCH 85)				
IPD072Aa	51 ± 30 (14–130)		49 ± 25 (15–93)	
PAT	25 ± 4.5 (18–37)		27 ± 4.3 (18–37)	
PMI	18 ± 2.5 (13–24)		18 ± 2.7 (12–25)	
Pollen (BBCH 63-65)				
IPD072Aa	1.6 ± 1.9 (0.34–9.7)		1.4 ± 0.85 (0.48–3.4)	
PAT	70 ± 7.8 (60–86)		71 ± 5.7 (61–83)	
PMI	31 ± 4.7 (20–39)		32 ± 4.6 (23–39)	

[a]Mean value.
[b]Standard deviation.
[c]Range.

3.3.5 | Inheritance and stability of inserted DNA

Genetic stability of maize DP51291 insert was assessed by Southern blot analysis on five generations (T1, T2, T3, T4 and T5) while inheritance pattern was assessed by quantitative polymerase chain reaction (qPCR)-based segregation analysis and phenotypic analysis (tolerance to glufosinate) from five generations (T1, T2, T3, T4 and T5). The results indicate that all the plants tested retained the single copy of the insert and flanking regions, which were stably inherited in subsequent generations.

The results support the presence of a single insertion, segregating in a Mendelian fashion.

3.3.6 | Conclusion on molecular characterisation

The molecular characterisation data establish that maize DP51291 contains a single insert consisting of one copy of the *ipd072Aa, mo-pat* and *pmi* expression cassettes. Bioinformatics analyses of the sequences encoding the newly expressed proteins and other ORFs within the insert or spanning the junctions between the insert and genomic DNA do not raise any safety concerns. 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 IPD072Aa, PAT and PMI proteins is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of plant and previously assessed microbe-produced IPD072Aa indicate that these proteins are equivalent (EFSA GMO Panel, 2024).

3.4 | Comparative analysis[10]

3.4.1 | Overview of studies conducted for the comparative analysis

Application GMFF-2021-0071 presents data on agronomic and phenotypic characteristics, as well as on forage and grain/seed composition of maize DP51291 (Table 2). In addition, the application contains data on characteristics of grain from maize DP51291.

TABLE 2 Main comparative analysis studies to characterise maize DP51291 provided in the application GMFF-2021-0071.

Study focus	Study details	Comparator	Non-GM reference varieties
Agronomic and phenotypic analysis	Field study, 10 sites in the US and 2 sites in Canada in 2021[a]	PHEJW/PHR03	20[b]
Compositional analysis	Field study, seven sites in the US and one site in Canada in 2021[a]		

Abbreviation: GM, genetically modified.

[a]The field trial sites in the US were in Iowa, Illinois, Indiana, Missouri, Nebraska, Pennsylvania and Texas; the site in Canada was in Ontario. Three additional sites were included for the agronomic and phenotypic analysis in US: Iowa, Illinois, Nebraska and one in Canada, Ontario.

[b]Non-GM hybrid maize with their corresponding comparative relative maturity indicated in brackets were 5433 (104); H3257 (104); K-0204 (104); P0574 (105); 205–17 (105); 207–27 (107); G07F23 (107); P0843 (108); P1093 (108); 5858 (108); H3394 (108); K-0608 (108); 209–50 (109); 6076 (110); 6046 (110); G10T63 (110); G11A33 (111); 6282 (112); G12W66 (112) and 6269 (112).

3.4.2 | Experimental field trial design and statistical analysis

At each field trial site, the following materials were grown in a randomised complete block design with four replicates: maize DP51291 not exposed to the intended herbicide (not treated), maize DP51291 exposed to the intended herbicide (treated), the comparator PHR03 × PHEJW and four non-GM reference varieties.

The agronomic, phenotypic and compositional data were analysed as specified by EFSA GMO Panel (2010b, 2011a). This includes, for each of the two treatments of maize DP51291, the application of a difference test (between the GM maize and the non-GM comparator) and an equivalence test (between the GM maize and the set of non-GM commercial reference varieties). The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).[11]

3.4.3 | Suitability of selected test materials

3.4.3.1 | *Selection of the test materials*

Inbred line PHR03 was transformed to obtain maize DP51291 (see Section 3.3.1 for the description of the transformation process), which was then crossed with the inbred line PHEJW to produce the hybrid maize DP51291 PHEJW/PHR03 used in the comparative analysis.

The comparator used in the field trials is the non-GM maize hybrid PHEJW/PHR03, which has a similar genetic background as maize DP51291 (as documented by the pedigree and the requested additional information), and is therefore considered to be the conventional counterpart.

Maize DP51291 and the conventional counterpart (PHEJW/PHR03), both with a comparative relative maturity (CRM) of 108, are appropriate for growing in environments across North America, where the comparative field trials were conducted.

[10]Technical dossier: Part II – Section 1.3; additional information: 31/7/2023.

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

Commercial non-GM reference varieties with a CRM ranging from 104 to 112 were selected by the applicant and, at each selected site, four reference varieties were tested (see Table 2). On the basis of the provided information on relative maturity classes, the GMO Panel considers the selected non-GM reference varieties acceptable for the comparative assessment.

3.4.3.2 | *Seed production and quality*

Seeds of maize DP51291 and the conventional counterpart used in the 2021 field trials were produced from plants harvested and stored under similar conditions, before being sown in the field trial sites. The seed lots were verified for their identity via event-specific qPCR analysis.

The grains were tested for their germination capacity under warm and cold temperature conditions.[12] Maize DP51291 and its conventional counterpart were compared for germination capacity and the results[13] of these studies indicate that the seed germination of maize DP51291 was not different than that of its conventional counterpart.

3.4.3.3 | *Conclusion on suitability*

The GMO Panel is of the opinion that the maize DP51291, 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.

3.4.4 | Representativeness of the receiving environments

3.4.4.1 | *Selection of field trial sites*

The selected field trial sites were located in commercial maize-growing regions of the United States and Canada. Climate and soil characteristics of the selected fields were diverse,[14] corresponding to optimal, near-optimal and sub-optimal conditions for maize cultivation (Sys et al., 1993). The GMO Panel considers that the selected sites, including the subset chosen for the compositional analysis, reflect commercial maize-growing regions in which the test materials are likely to be grown.

3.4.4.2 | *Meteorological conditions*

Maximum and minimum mean temperatures and sum of precipitations were provided on a daily basis. Some exceptional weather conditions were reported at three of the selected sites.[15] However, due to the lack of major impacts on plant growth at these sites, the GMO Panel considers that the exceptional weather conditions did not invalidate the selection of the field trial sites for the comparative analyses.

3.4.4.3 | *Management practices*

The field trials included plots containing maize DP51291, plots with the conventional counterpart and plots with non-GM reference varieties, managed according to local agricultural practices. In addition, the field trials included plots containing the GM maize managed following the same agricultural practices, but conventional herbicides were replaced with the intended glufosinate ammonium-containing herbicide. The intended herbicide was applied at the BBCH 14–15 growth stage.[16] The GMO Panel considers that the management practices, including sowing, harvesting and application of plant protection products, were appropriate for the field trials.

3.4.4.4 | *Conclusion on representativeness*

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

[12]Warm temperature condition corresponds to 25°C for 7 days and cold temperature to 10°C for 7 days followed by 5 days at 25°C.

[13]The GM hybrid maize and its conventional counterpart both showed a mean germination of 99% and 98% under warm and cold temperature conditions respectively.

[14]Soil types of the field trials were clay, clay loam, loam, loamy sand, sand, sandy clay loam, sandy loam, silt loam and silty clay loam. Soil organic carbon ranged from 0.7% to 2.5%; pH ranged from 5.2 to 8.2; average temperatures and sum of precipitations during the usual crop growing season ranged respectively from 17.1 to 26.0°C and from 236 to 834 mm.

[15]Windstorm was recorded at one field trial in Iowa and at one in Missouri. Hail and windstorm were recorded at one field trial in Texas.

[16]BBCH scale describes phenological stages (Meier, 2001)

3.4.5 | Agronomic and phenotypic analysis

Eleven agronomic and phenotypic endpoints[17] plus information on abiotic stressors, disease incidence and arthropod damage were collected from the field trials (see Table 2). Three endpoints (lodging, ear count and dropped ears) were not analysed with formal statistical methods because of lack of variability in the data.

The statistical analysis (Section 3.4.2) was applied to eight endpoints, with the following results:

- For maize DP51291 (not treated), the test of difference identified a statistically significant difference with the conventional counterpart for days to flowering, which fell under equivalence category I.
- For maize DP51291 (treated), the test of difference identified statistically significant differences with the conventional counterpart for days to flowering, which fell under equivalence category I.

3.4.6 | Compositional analysis

Maize DP51291 grain and forage harvested from eight sites (Table 2) were analysed for 80 constituents (10 in forage and 70 in grains), including those recommended by OECD (OECD, 2002). The statistical analysis was not applied to 11 grain constituents because their concentration in more than half of the samples were below the limit of quantification.[18]

The statistical analysis was applied to a total of 69 constituents (59 in grain[19] and 10 in forage[20]); a summary of the outcome of the test of difference and the test of equivalence is presented in Table 3:

- For maize DP51291 not treated with the intended herbicide, statistically significant differences with the conventional counterpart were found for 13 endpoints (all in grain). All these endpoints for which significant differences were found between the GM maize and the conventional counterpart fell under equivalence category I or II, except for manganese in grain which fell under equivalence category III and oleic acid (C18:1) and proline in grain which fell under equivalence category IV. The equivalence test could not be done for sodium because of the lack of variation among the non-GM reference varieties.
- For maize DP51291 treated with the intended herbicide, statistically significant differences with the conventional counterpart were found for 19 endpoints (four in forage and 15 in grain). All these endpoints for which significant differences were found between the GM maize and the conventional counterpart fell under equivalence category I or II, except for phosphorus in forage and for linoleic acid (C18:2) and manganese in grain which fell under equivalence category III and for oleic acid (C18:1) in grain which fell under equivalence category IV. The equivalence test could not be done for sodium because of the lack of variation among the non-GM reference varieties.

TABLE 3 Outcome of the comparative compositional analysis in grain and forage for maize DP51291. The table shows the number of endpoints in each category.

		Test of difference[a]			
		Treated[c]		Not-treated[c]	
		Not different	Significantly different	Not different	Significantly different
Test of equivalence[b]	Category I/II	38	15[d]	41	10[d]
	Category III/IV	11[e]	4[f]	14[e]	3[f]
	Not categorised	1[g]	–	1[g]	–
	Total endpoints	69		69	

(Continues)

[17]Early stand count, days to flowering, plant height, days to maturity, lodging, final stand count, ear count, dropped ears, yield, harvest grain moisture and 100-kernel weight.
[18]Lauric acid (C12:0), myristic acid (C14:0), heptadecanoic acid (C17:0), heptadecenoic acid (C17:1), eicosadienoic acid (C20:2), behenic acid (C22:0), riboflavin, β-tocopherol, δ-tocopherol, furfural and raffinose.
[19]Proximate and fibre content (ash, carbohydrates, crude fat, crude fibre, crude protein, moisture, acid detergent fibre (ADF), neutral detergent fibre (NDF) and crude fibre), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc), vitamins (β-carotene, α-tocopherol, γ-tocopherol, total tocopherols, niacin, pantothenic acid, pyridoxine, thiamine, folic acid), amino acids (alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine), fatty acids (palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), α-linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C20:1) and lignoceric acid (C24:0)) and other compounds (p-coumaric acid, ferulic acid, inositol, phytic acid and trypsin inhibitor).
[20]Moisture, crude protein, crude fat, ash, carbohydrates, crude fibre, acid detergent fibre (ADF), neutral detergent fibre (NDF), calcium and phosphorus.

TABLE 3 (Continued)

[a]Comparison between maize DP51291 and its conventional counterpart.

[b]Four different outcomes: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence). Not categorised means that the test of equivalence was not applied because of the lack of variation among the non-GM reference varieties.

[c]Treated/not treated with the intended herbicide.

[d]Endpoints with significant differences between maize DP51291 and its conventional counterpart falling in equivalence category I–II. For forage, treated only: crude protein, moisture and carbohydrates. For grains, not treated only: stearic acid (C18:0), thiamine, folic acid, pyridoxine; treated only: moisture, crude fat, tyrosine, pantothenic acid, a-tocopherol, trypsin inhibitor; both treated and not treated: palmitic acid (C16:0), palmitoleic acid (C16:1), eicosenoic acid (C20:1), lignoceric acid (C24:0), copper, ferulic acid.

[e]Endpoints in seeds and forage with no significant differences between maize DP51291 and its conventional counterpart and falling under equivalence category III: For grains, not treated only: alanine, aspartic acid, serine, phytic acid, linoleic acid (C18:2); treated only: carbohydrates, proline; both treated and not treated: crude protein, magnesium, glutamic acid, histidine, isoleucine, leucine, phenylalanine, threonine, valine.

[f]Endpoints with significant differences between the maize DP51291 and its conventional counterpart and falling in equivalence category III–IV. For forage, treated only: phosphorus. For grains, not treated only: proline; treated only: linoleic acid (C18:2); both treated and not treated: manganese and oleic acid (C18:1). Estimated means are reported for these endpoints in Table 4.

[g]Endpoints not categorised for equivalence and without significant differences between the maize DP51291 and its conventional counterpart: in grain, both treated and not treated: sodium.

The GMO Panel assessed all significant differences between maize DP51291 and its conventional counterpart, taking into account potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties. Quantitative results for the endpoints showing significant differences between maize DP51291 and its conventional counterpart and falling under category III/IV are given in Table 4.

TABLE 4 Quantitative results (estimated means and equivalence limits) for compositional endpoints in grains and forage that are further assessed based on the results of the statistical analysis.

		Maize DP51291[a]		Conventional counterpart	Non-GM reference varieties	
	Endpoint	Not treated	Treated		Mean	Equivalence limits
Grains	Manganese (% dw)	0.000790*	0.000792*	0.000822	0.000626	0.000466–0.000787
	Proline (% dw)	1.02*	1.01	0.996	0.844	0.753–0.934
	Oleic acid (C18:1) (% FA)	22.2*	22.4*	21.9	28.8	23.6–34
	Linoleic acid (C18:2) (% FA)	59.8	59.6*	60.0	54.2	48.9–59.5
Forage	Phosphorus (% dw)	0.269	0.256*	0.271	0.298	0.265–0.331

[a]For the maize DP51291, significantly different values are marked with an asterisk, while the outcomes of the test of equivalence are differentiated by greyscale backgrounds: white (equivalence category I or II), light grey (equivalence category III) and dark grey (equivalence category IV). Treated: treated with the intended herbicide; not treated: treated only with conventional herbicides (see Section 3.4.4.3).

Abbreviations: % FA, percentage total fatty acids; dw, dry weight.

3.4.7 | Conclusion on comparative analysis

Considering the selection of test materials, the field trial sites and the associated management practices and the agronomic-phenotypic characterisation as an indicator of the overall field trial quality, the GMO Panel concludes that the field trials are appropriate to support 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 agronomic and phenotypic characteristics between maize DP51291 and its conventional counterpart needs further assessment regarding their potential environmental impact.
- None of the differences identified in forage and grain composition between maize DP51291 and its conventional counterpart needs further assessment regarding food and feed safety except for phosphorus in forage and manganese, proline, oleic acid (C18:1) and linoleic acid (C18:2) in grain, which are further assessed in Section 3.5.

3.5 | Food/feed safety assessment[21]

3.5.1 | Overview of overarching information for food/feed assessment

3.5.1.1 | *Compositional analysis*

The compositional analysis of maize DP51291 and its conventional counterpart provided by the applicant and assessed by the GMO Panel is described in Section 3.4.6.

[21]Dossier: Part II – Section 1.4; additional information: 21/11/2023 and 29/2/2024.

3.5.1.2 | *Newly expressed proteins*

Three proteins, IPD072Aa, PAT and PMI, are newly expressed in maize DP51291. The three proteins have been previously assessed by the GMO Panel (EFSA GMO Panel, 2024) and no safety concerns for humans and animals were identified. No new studies have been provided in the context of this application.

3.5.1.2.1 | *Molecular characterisation*

The protein characterisation of the newly expressed IPD072Aa, PAT and PMI proteins provided by the applicant and assessed by the GMO Panel is described in Section 3.3.3.

3.5.1.2.2 | *Synergistic or antagonistic interactions*

The potential for a functional interaction among the IPD072Aa, PAT and PMI proteins has been assessed with regard to human and animal health. Based on current scientific knowledge on the mode of action of the three proteins (Table 5), no synergistic or antagonistic interactions between these three proteins, which could raise safety concerns for food and feed from maize DP51291, are expected.

TABLE 5 Intended effect and mode of action of the NEPs in maize DP51291.

Protein	Intended effect in GM plant and mode of action
IPD072Aa	The IPD072Aa protein confers resistance to certain coleopteran pests when expressed in plants by causing disruption of their midgut epithelium
PAT	The PAT protein confers tolerance to glufosinate ammonium-containing herbicides by acetylation of glufosinate ammonium
PMI	The PMI protein was used as a selectable marker because cells can utilise mannose as a carbon source. PMI catalyses the isomerisation of mannose-6-phosphate to fructose-6-phosphate (Negrotto et al., 2000)

3.5.1.3 | *Effect of processing*

Maize DP51291 will undergo existing production processes used for conventional maize. No novel production process is envisaged. Based on the outcome of the comparative assessment, processing of the GM maize into food and feed products is not expected to result in products being different from those of conventional non-GM maize varieties.

3.5.2 | Toxicology

The strategies to assess the toxicological impact of any changes on the whole genetically modified food and feed resulting from the genetic modification focus on the assessment of (i) newly expressed proteins; (ii) new constituents other than NEPs; (iii) altered levels of food and feed constituents; and (iv) the whole genetically modified food and feed.

3.5.2.1 | *Testing of newly expressed proteins*

The IPD072Aa, PAT and PMI proteins were previously assessed by the GMO Panel in the context of other applications (EFSA GMO Panel, 2024) and no safety concerns for humans and animals (i.e. farmed and companion animals) were identified. Updated bioinformatics analyses revealed no similarities of the IPD072Aa, PAT and PMI proteins with known toxins. The GMO Panel is not aware of any new information that would change the previous conclusion on the safety of the IPD072Aa, PAT and PMI proteins.

3.5.2.2 | *Assessment of new constituents other than proteins*

Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, no new constituents other than the newly expressed proteins have been identified in grains and forage from maize DP5129. Therefore, no further food and feed safety assessment of components other than the newly expressed proteins is required.

3.5.2.3 | *Assessment of altered levels of food and feed constituents*

No altered levels of food/feed constituents have been identified in grains and forage maize DP5129 except for phosphorus in forage and manganese, proline, oleic acid (C18:1) and linoleic acid (C18:2) in grain. These changes are considered not to represent a toxicological concern, considering the biological role of the affected constituent and the magnitude of the

changes, therefore, no further toxicological assessment is needed. Further information on the relevance of these findings is provided in Section 3.5.5.

3.5.2.4 | *Testing of the whole genetically modified food and feed*

Based on the outcome of the molecular characterisation, comparative analysis and toxicological assessment, no indications of findings relevant to food and feed safety have been identified for maize DP51291 related to the stability and expression of the insert, and to modifications of toxicological concern in the composition of maize DP51291 (see Sections 3.4.1 MC, 3.4.2 Compo and 3.4.3.3 tox). Therefore, animal studies with food/feed derived from maize DP51291 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 fed with diets containing grains/meal (toasted and defatted) derived from maize DP51291.

In this study, pair-housed Crl:CD (SD) rats (16 per sex per group; 2 rats per cage) were allocated to six groups using a randomised complete block design with eight replications per sex.

Groups were fed diets containing maize DP51291 grains from plants treated with the intended herbicide (glufosinate) at 50% and 33% of inclusion level (the latter supplemented with 17% of the non-GM comparator maize), the non-GM comparator (inclusion level 50%) and the three reference varieties (P0843, BK5883, P0760) (inclusion level 50%).

The study was adapted from OECD test guideline 408 (OECD, 2018), aligned with EFSA Scientific Committee guidance (EFSA Scientific Committee, 2011) and complied with the principles of good laboratory practice (GLP) with some minor deviations described in the study report, not impacting the study results and interpretation.

The stability of the test and control materials was not analytically verified; however, it was confirmed that the diet was used in accordance to product expiration declared by the diet manufacturer. The GMO Panel considered this acceptable evidence that the constituents of the diets would be stable for the duration of the treatment. Furthermore, 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.

Event-specific PCR analysis confirmed the presence of the maize DP51291 in both the GM grains and diets and excluded the presence of the event in the respective controls. ELISA analyses also confirmed the presence of the maize DP51291 in the GM maize grains and GM diets.

Both the GM grains and diets were analysed for nutrients, antinutrients and potential contaminants. Balanced diets were formulated based on the specifications for PMI Certified Rodent LabDiet® #5002.

Feed and water were provided ad libitum. In-life procedures and observations and terminal procedures were conducted in accordance to OECD TG 408 (2018).

An appropriate range of statistical tests were performed on the results of the study. Detailed description of the methodology and of statistically significant findings identified in rats given diets containing grains/meal derived from maize DP51291 is reported in Appendix C.

There were no test diet-related incidents of mortality or clinical signs. One male rat from the DP5129 low dose group was found dead on test day 26, but it is not considered treatment-related. No test diet-related adverse findings were identified in any of the investigated parameters. A small number of statistically significant findings were noted but these were not considered adverse effects of treatment for one or more of the following reasons:

- were within the normal variation[22] for the parameter in rats of this age;
- were of small magnitude;
- were identified at only a small number of time intervals with no impact on the overall value;
- exhibited no consistent pattern with related parameters or endpoints;
- exhibited no consistency with increasing incorporation levels.

No gross pathology findings related to the administration of the test diet were observed at necropsy, and the microscopic examinations of a wide range of organs and tissues did not identify relevant differences in the incidence or severity of the histopathological findings related to the administration of the test diet compared to the control group.

The GMO Panel concludes that this study is in line with the requirements of Regulation (EU) No 503/2013 and that no treatment-related adverse effects were observed in rats after feeding diets containing maize DP51291 grains at 33% or 50% for 90 days.

[22]Although animals used in a toxicology study are of the same strain, from the same supplier and are closely matched for age and body weight at the start of the study, they exhibit a degree of variability in the parameters investigated during the study. This variability is evident even within control groups. To help reach a conclusion on whether a statistically significant finding in a test group is treatment-related, account is taken of whether the result in the test group is outside the normal range for untreated animals of the same strain and age. To do this, a number of sources of information are considered, including the standardised effect size, the standard deviations and range of values within test and control groups in the study and, if applicable, data from other studies performed in the same test facility within a small timeframe and under almost identical conditions (Historic Control Data).

3.5.3 | Allergenicity

The strategies to assess the potential risk of allergenicity focus: (i) on the source of the recombinant protein; (ii) on the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons; and (iii) on whether the transformation may have altered the allergenic properties of the modified plant. Furthermore, the assessment also takes into account potential adjuvant properties of the newly expressed proteins, which is defined as the ability to enhance an allergic reaction.

3.5.3.1 | *Assessment of allergenicity of the newly expressed proteins*

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 yielded sufficient evidence to predict allergenicity (Codex Alimentarius, 2009; EFSA GMO Panel, 2011a, 2017; Regulation (EU) No 503/2013).

The *ipd072Aa, mo-pat and pmi* genes originate from *P. chlororaphis*, *S. viridochromogenes* and *E. coli*, respectively, none of which are considered common allergenic sources.

Updated bioinformatic analyses of the amino acid sequences of the IPD072Aa, PAT and PMI proteins, using the criterion of 35% identity in a sliding window of 80 amino acids, revealed no significant similarities to known allergens.

The GMO Panel has previously evaluated the safety of the IPD072Aa, PAT and PMI proteins and no concerns for allergenicity were identified (e.g. EFSA GMO Panel, 2024). The GMO Panel is not aware of any new information that would change this conclusion.

In addition, the GMO Panel did not find an indication that the newly expressed proteins IPD072Aa, PAT and PMI at the levels expressed in maize DP51291 might be adjuvants.

The applicant also provided information on the safety of the IPD072Aa, PAT and PMI proteins regarding their potential to cause a celiac disease response. For this assessment, the applicant followed the principles described in the EFSA GMO Panel guidance document (EFSA GMO Panel, 2017). The assessment of the IPD072Aa protein identified no perfect or relevant partial matches with known celiac disease peptide sequences. The assessment of the PAT and PMI proteins revealed partial matches containing the Q/E-X1-P-X2 motif. These partial matches were previously assessed by the EFSA GMO Panel (2024) and no safety concerns were identified.

The GMO Panel considers that there are no indications that the newly expressed IPD072Aa, PAT and/or PMI proteins in maize DP51291 may be allergenic.

3.5.3.2 | *Assessment of allergenicity of the whole GM plant or crop*

The GMO Panel regularly reviews the available publications on food allergy to maize. However, maize is not considered a common allergenic food[23] (OECD, 2002). Therefore, the GMO Panel does not request experimental data to analyse the allergen repertoire of GM maize. In the context of this application and considering the data from the molecular characterisation, the compositional analysis and the assessment of the newly expressed proteins (see Sections 3.3, 3.4 and 3.5), the GMO Panel identifies no indications of potentially increased allergenicity of food and feed derived from GM maize DP51291 compared with that derived from its conventional counterpart and the non-GM reference varieties tested.

3.5.4 | Dietary exposure assessment to new constituents

In line with Regulation (EU) No 503/2013 the applicant provided dietary exposure estimates to IPD072Aa, PAT and PMI proteins newly expressed in maize DP51291. Dietary exposure was estimated based on protein expression levels reported in this application for maize DP51291, the current available consumption data and feed practices, the foods and feeds currently available in the market and the described processing conditions.

For the purpose of estimating dietary exposure, the levels of newly expressed proteins in DP51291 maize grains, forage and pollen were derived from replicated field trials (four replicates from six locations) in the United States and Canada during the 2021 growing season (Table 1 in Section 3.3.4).

3.5.4.1 | *Human dietary exposure*

Chronic and acute dietary exposure to IPD072Aa, PAT and PMI proteins newly expressed in maize DP51291 were provided. The applicant followed the methodology described in the EFSA Statement 'Human dietary exposure assessment to newly expressed protein in GM foods' to anticipate human dietary exposure making use of summary statistics of consumption (EFSA, 2019a).

[23]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.

Human dietary exposure was estimated across European countries for 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 in DP51291 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).[24] Corn oil, corn starch and corn syrup were excluded from the assessment since no proteins are expected to be present in these commodities.

Mean protein expression values on fresh weight basis 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, 2019a). Different recipes and factors were considered to estimate the amount of maize in the consumed commodities before assigning newly expressed protein levels to the relevant commodities.[25] No losses in the newly expressed proteins during processing were considered, except for the commodities mentioned above.

The highest anticipated acute dietary exposure (highly exposed population) was estimated in the age class 'Other children' with estimates of 48.6, 108 and 91.2 µg/kg bw per day for IPD072Aa, PAT and PMI proteins, respectively. The main contributor to the exposure in the dietary survey with the highest estimates would be corn grains.

The highest anticipated chronic dietary exposure (highly exposed population) was estimated in the age class 'Infants' with estimates of 26.2, 58.0 and 49.1 µg/kg bw per day for IPD072Aa, PAT and PMI proteins, respectively. The main contributor to the exposure in the dietary survey with the highest estimates would corn flakes.

An ad hoc dietary exposure scenario was provided for consumers of pollen supplements under the assumption that these supplements might be made of pollen from DP51291 maize. Consumption data on pollen supplements are available for few consumers across seven different European countries.[26] The low number of consumers available adds uncertainty to the exposure estimations which should be carefully interpreted, and only allows the estimation of dietary exposure for average consumers. The highest mean acute dietary exposure would range from 0.98 µg/kg bw per day for IPD072Aa to 49.5 µg/kg bw per day for PAT, in the elderly population. Similarly, the highest mean chronic dietary exposure in consumers of pollen supplements would range from 0.65 µg/kg bw per day for IPD072Aa to 33.0 µg/kg bw per day for PAT, also in the elderly population.

3.5.4.2 | *Animal dietary exposure*

Anticipated dietary exposure to IPD072Aa, PAT and PMI proteins in maize DP51291 was estimated across different animal species, as described below, assuming the consumption of maize products commonly entering the feed supply chain (i.e. maize grains and forage). A conservative scenario with 100% replacement of conventional maize products by the maize DP51291 products was considered.

Mean levels (dry weight) of the newly expressed proteins in grains and forage from maize DP51291 treated with the intended herbicide used for animal dietary exposure are listed in Table 1 in Section 3.3.4.

The applicant estimated dietary exposure to IPD072Aa, PAT and PMI proteins in livestock (i.e. poultry, swine, cattle and sheep), based on estimates for body weights, daily feed intakes and inclusion rates (percentage) of maize grains and forage in rations (OECD, 2013). Estimated dietary exposure in livestock animals was calculated based on the consumption of maize grain and forage alone or in combination, as reported in Appendix D (Tables D.1–D.3).

3.5.5 | Nutritional assessment of endogenous constituents

The intended traits of maize DP51291 are herbicide tolerance and insect resistance, with no intention to alter nutritional parameters. However, levels of phosphorus in forage (treated), and manganese (both treated and not treated), proline (not treated), oleic acid (C18:1) (both treated and not treated) and linoleic acid (C18:2) (treated) in grain were significantly different from its conventional counterpart and showed a lack of equivalence with the set of non-GM reference varieties (Section 3.4.6). The biological relevance of these compounds, the role of maize as contributor to their total intake and the magnitude and direction of the observed changes were considered during the nutritional assessment.

3.5.5.1 | *Human nutrition*

Manganese is an essential dietary mineral which is a component of a number of metalloenzymes involved in amino acid, lipid and carbohydrate metabolism. Considering that a specific manganese deficiency syndrome has not been described in humans (EFSA NDA Panel, 2013), the decrease of ~4% observed for both treated and not treated maize DP51291 compared to its conventional counterpart does not represent a nutritional concern.

[24] https://www.efsa.europa.eu/en/applications/gmo/tools. EFSA consumption database: version 1.0 (updated March 2022).

[25] Example: 100 g of maize bread are made with approximately 74 g of maize flour, and a reverse yield factor of 1.22 from the conversion of maize grains into flour is used. This results in ~6.4 µg of PAT per gram of maize bread as compared to the 7.1 µg/g reported as mean concentration in the maize grains.

[26] Example: 100 g of maize bread are made with approximately 74 g of maize flour, and a reverse yield factor of 1.22 from the conversion of maize grains into flour is used. This results in ~6.4 µg of PAT per gram of maize bread as compared to the 7.1 µg/g reported as mean concentration in the maize grains.

Proline is a dispensable amino acid as it can be synthesised in the human body at a rate to meet the metabolic requirement (EFSA NDA Panel, 2012). The increase of ~2% observed in the not treated maize DP51291 as compared to the conventional counterpart poses no nutritional concern.

A small increase of ~2% of oleic acid in both treated and not treated compared to its conventional counterpart was reported. No dietary reference values (DRVs) for cis-monounsaturated fatty acids are proposed by EFSA since they are synthesised by the body (EFSA NDA Panel, 2010). Therefore, the observed change does not represent a nutritional concern.

As regards to linoleic acid, a slight decrease of ~1% was observed for treated maize DP51291 compared to its conventional counterpart. An adequate intake (AI) for linoleic acid of 4 energy % has been proposed (EFSA NDA Panel, 2010). Although linoleic acid is the most abundant fatty acid in maize, considering the magnitude of the decrease, the GMO Panel identified no nutritional concern.

3.5.5.2 | *Animal nutrition*

Diets for animals are usually balanced for the content of major minerals, including phosphorus and manganese, and, eventually, supplemented when the amount provided by feed is not enough to satisfy nutritional requirements.

Phosphorus in cereals is mainly bound to phytic acid, largely reducing its bioavailability especially in non-ruminant animals. The observed decrease level in treated GM maize forage compared to its comparator does not pose an issue for animals, since complete diets are balanced with mineral premixes.

Manganese is an important trace element in animal nutrition. The observed decrease level in treated and not-treated GM maize grains compared to its comparator does not constitute an issue for animals, since complete diets are balanced with mineral premixes. Moreover, maize grains are also considered a poor source of manganese (McDonald et al., 2011).

Diets for animals are usually balanced for the content of fatty acids, and eventually supplemented when the amount provided by feed is not enough to satisfy nutritional requirements.

Oleic acid (C18:1) is a non-essential fatty acid. The observed increase level in treated and not-treated GM maize grains compared to its comparator does not represent a concern for animal nutrition.

Linoleic acid (C18:2 n-6) is an essential fatty acid, because it cannot be synthetized in the body, and must be supplied in diets. It is the most dominant fatty acid in maize grain, and the observed decrease level in treated GM maize grains compared to its comparator is not an issue for animal nutrition. Although the differences were statistically significant, the effect size was small and of little practical significance.

Proline is an aromatic and heterocyclic amino acid, and animals synthesise proline from arginine and glutamine/glutamate. The relative increase of ~2% observed in the not treated maize DP51291 as compared to the conventional counterpart poses no nutritional concern.

3.5.6 | Post-market monitoring of GM food/feed

Maize DP51291, as described in this application, does not raise any nutritional concern and is as safe as its conventional counterpart and the non-GM reference varieties tested. The GMO Panel concludes that based on the information considered in its safety assessment, a post-market monitoring plan for food and feed is not necessary.

3.5.7 | Conclusions on the food/feed safety assessment

The proteins IPD072Aa, PAT and PMI newly expressed in maize DP51291 do not raise safety concerns for human and animal health. No interactions between the newly expressed proteins relevant for food and feed safety were identified. Moreover, the GMO Panel did not identify indications of safety concerns regarding allergenicity or adjuvanticity related to the presence of the newly expressed proteins in maize DP51291. The GMO Panel finds no evidence that the genetic modification impacts the overall safety of maize DP51291. Based on the outcome of the comparative assessment and the nutritional assessment, the GMO Panel concludes that the consumption of maize DP51291 does not represent any nutritional concern, in the context of the scope of this application. The GMO Panel concludes that maize DP51291, as described in this application, is as safe as the conventional counterpart and the non-GM reference varieties tested, and no post-market monitoring of food/feed is considered necessary.

3.6 | Environmental risk assessment and monitoring plan[27]

3.6.1 | Environmental risk assessment

Considering the scope of application GMFF-2021-0071, which excludes cultivation, the environmental risk assessment (ERA) of maize DP51291 mainly takes into account: (1) the exposure of microorganisms to recombinant DNA in the gastrointestinal

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tract of animals fed with 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 GM material, including viable maize DP51291 grains, during transportation and/or processing (EFSA GMO Panel, 2010a).

3.6.1.1 | *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. Survival is limited mainly by a combination of low competitiveness, absence of a dormancy phase and susceptibility to plant pathogens, herbivores and cold climate conditions (OECD, 2003), even though occasional feral GM maize plants may occur outside cultivation areas in the EU (e.g. Pascher, 2016). Field observations indicate that maize grains may survive and overwinter in some EU regions, resulting in volunteers in subsequent crops (e.g. Gruber et al., 2008; Palaudelmàs et al., 2009; Pascher, 2016). However, maize volunteers have been shown to grow weakly and flower asynchronously with the maize crop (Palaudelmàs et al., 2009). Thus, the establishment and survival of feral and volunteer maize in the EU is currently limited and transient.

It is unlikely that the intended traits of maize DP51291 will provide a selective advantage to maize plants, except when they are exposed to glufosinate-containing herbicides or infested by insect pests that are susceptible to the IPD072Aa proteins. However, if this was to occur this fitness advantage will not allow the GM plant 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 is very unlikely that maize DP51291 will differ from conventional maize hybrid varieties in their 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 DP51291 grains.

3.6.1.2 | *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 animals, and in other environments, may be exposed to fragments of DNA, including the recombinant fraction of such DNA.

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

Homologous recombination is known to facilitate horizontal transfer of non-mobile, chromosomal DNA fragments to bacterial genomes. 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 (EFSA, 2009; Hülter & Wackernagel, 2008). 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.

Bioinformatic analysis of event DP51291 revealed that sufficient sequence identity was detected with the *pmi* coding sequence from *E. coli*. No paired alignments and, thus, no potential to facilitate double HR were identified. Gene replacements of *pmi* sequence on natural *E. coli* might potentially occur in the main receiving environments, i.e. the gastrointestinal tract, but this would not confer any new trait or selective advantage to bacterial recipients. The analysis also confirmed that the genetic elements encoding for PAT and IPD072Aa proteins were plant codon-optimised and did not provide sufficient sequence identity to bacterial DNA.

In summary, 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 maize DP51291 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 & Sweet, 2002; EFSA, 2016, 2022; OECD, 2003; 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, 2022; Le Corre et al., 2020; Trtikova et al., 2017).

The potential of spilled maize grains to establish, grow and produce pollen is extremely low and transient (see Section 3.6.1.1). Therefore, the likelihood/frequency of cross-pollination between occasional feral GM maize plants resulting from grain spillage, and weedy or cultivated *Zea* plants is considered extremely low (EFSA, 2016, 2022). 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 3.6.1.1.

3.6.1.3 | *Interactions of the GM plant with target organisms*

Taking the scope of application GMFF-2021-0071 into account (no cultivation), potential interactions of occasional feral maize DP51291 plants arising from grain import spills with the target organisms are not considered a relevant issue.

3.6.1.4 | *Interactions of the GM plant with non-target organisms*

The GMO Panel evaluated the potential hazards of the NEPs and considered that the environmental exposure of non-target organisms to spilled GM maize material or occasional feral GM maize plants arising from spilled maize DP51291 grains will be limited. Additionally, ingested proteins are typically degraded before entering the environment through faecal material of animals fed with GM maize (Harmon & Swanson, 2020; Miner-Williams et al., 2014; Mok & Urschel, 2020; Santos-Hernández et al., 2018; van Bruchem et al., 1985), and the data provided for the assessment of protein stability (see Section 3.5.1.2; EFSA GMO Panel, 2024) supports that also the NEPs will be degraded. Based on the comparative approach the GMO Panel considers that potential interactions of maize DP51291 with non-target organisms do not raise any environmental safety concern.

3.6.1.5 | *Interactions with abiotic environment and biogeochemical cycles*

The GMO Panel evaluated the potential hazards of the NEPs and considered that the environmental exposure to spilled GM maize material or occasional feral GM maize plants arising from spilled maize DP51291 grains will be limited. Additionally, ingested proteins are typically degraded before entering the environment through faecal material of animals fed with GM maize (Harmon & Swanson, 2020; Miner-Williams et al., 2014; Mok & Urschel, 2020; Santos-Hernández et al., 2018; van Bruchem et al., 1985), and the data provided for the assessment of protein stability (see Section 3.5.1.2; EFSA GMO Panel, 2024) supports that also the NEPs will be degraded. Based on the comparative approach the GMO Panel considers that potential interactions of maize DP51291 with the abiotic environment and biogeochemical cycles do not raise any environmental safety concern.

3.6.2 | Post-market environmental monitoring

The objectives of a post-market environmental monitoring (PMEM) plan, according to Annex VII of Directive 2001/18/EC, are: (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the ERA are correct; and (2) to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment that were not anticipated in the ERA.

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

As the ERA did not identify potential adverse environmental effects from maize DP51291, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for maize DP51291 includes: (1) the description of a monitoring approach involving operators (federations involved in import and processing), reporting to the applicant, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment; (2) a coordinating system established by CropLife Europe for the collection of information recorded by the various operators; and (3) the review of relevant scientific publications retrieved from literature searches (Lecoq et al., 2007; Windels et al., 2008). The applicant proposes to submit a PMEM report on an annual basis for the duration 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 DP51291. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

3.6.2.1 | *Conclusion of the environmental risk assessment and monitoring plan*

The GMO Panel concludes that it is unlikely that maize DP51291 would differ from conventional maize varieties in its ability to persist under European environmental conditions. Considering the scope of application GMFF-2021-0071, interactions of occasional feral maize DP51291 plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from maize DP51291 to bacteria does not indicate a safety concern. Therefore, considering the introduced traits, the outcome of the agronomic and phenotypic analysis, and the routes and levels of exposure, the GMO Panel concludes that maize DP51291 would not raise safety concerns in the event of accidental release of GM material, including 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 DP51291.

4 | OVERALL CONCLUSIONS

The GMO Panel was asked to carry out a scientific assessment of maize DP51291 for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003. The molecular characterisation data establish that maize DP51291 contains a single insert consisting of one copy of the *pmi*, *mo-pat* and *ipd072Aa* expression cassettes. The quality of the sequencing methodology and datasets was assessed by the EFSA GMO Panel and is in compliance with the requirements listed in the EFSA Technical Note (2018). Bioinformatics analyses of the sequences encoding the newly expressed protein and other ORFs present within the insert or spanning the junctions between the insert and genomic DNA, do not raise any safety concerns. The stability of the inserted DNA and of the introduced trait is confirmed over several generations. The methodology used to quantify the levels of the PMI, PAT and IPD072Aa proteins is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of plant and previously assessed microbe-produced IPD072Aa indicate that these proteins are equivalent.

Considering the selection of test materials, the field trial sites and the associated management practices and the agronomic-phenotypic characterisation as an indicator of the overall field trial quality, the GMO Panel concludes that the field trials are appropriate to support the comparative analysis. None of the identified differences in the agronomic/phenotypic and compositional characteristics tested between maize DP51291 and its conventional counterpart needed further assessment, except for phosphorus in forage and manganese, proline, oleic acid (C18:1) and linoleic acid (C18:2) in grain which do not raise nutritional and safety concerns. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the PMI, PAT and IPD072Aa proteins as expressed in maize DP51291, and finds no evidence that the genetic modification impacts the overall allergenicity of maize DP51291. In the context of this application, the consumption of food and feed from maize DP51291 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that maize DP51291 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 maize DP51291 material into the environment, this would not raise environmental safety concerns. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of maize DP51291. 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 DP51291.

The GMO Panel concludes that maize DP51291 is as safe as its conventional counterpart and the tested non-GM maize reference varieties with respect to potential effects on human and animal health and the environment.

5 | DOCUMENTATION AS PROVIDED TO EFSA

- Letter from the Competent Authority of The Netherlands received on 30 January 2023 concerning a request for authorisation of the placing on the market of genetically modified maize DP51291, submitted in accordance with Regulation (EC) No 1829/2003 by Corteva Agriscience (EFSA Ref. GMFF-2021-0071; EFSA-Q-2023-00051)
- The application was made valid on 4 May 2023
- Additional information (1) was requested on 31 May 2023
- Additional information (1) was received on 31 July 2023
- Additional information (2) was requested on 10 August 2023
- Additional information (2) was received on 29 September 2023
- Additional information EURL (3) was requested on 15 September 2023
- Additional information (3) was received on 10 November 2023
- Additional information (4) was requested on 21 November 20203
- Additional information (4) was received on 17 January 2024 partial; on 26 January 2024 complete
- Additional information (5) was requested on 29 February 2024
- Additional information (5) was received on 2 April 2024
- Additional information (6) was requested on 10 April 2024
- Additional information (6) was received on 2 May 2024

- Additional information (7) was requested on 8 May 2024
- Additional information (7) was received on 6 August 2024
- Additional information (8) was requested on 24 July 2024
- Additional information (8) was received on 18 September 2024

ABBREVIATIONS

ADF	acid detergent fibre
bp	base pair
bw	body weight
CaMV	cauliflower mosaic virus
dw	dry weight
ELISA	enzyme-linked immunosorbent assay
ERA	environmental risk assessment
fw	fresh weight
GLP	good laboratory practice
GM	genetically modified
GMO Panel	EFSA Panel on Genetically Modified Organisms
GMO	genetically modified organism
HGT	horizontal gene transfer
HR	homologous recombination
JSA	junction sequence analysis
MS	mass spectrometry
NDF	neutral detergent fibre
NEP	newly expressed protein
OECD	Organisation for Economic Co-operation and Development
ORFs	open reading frames
PAT	phosphinothricin acetyltransferase
PCR	polymerase chain reaction
PMEM	post-market environmental monitoring
PMI	phosphomannose isomerase
SbS	Southern-by-sequencing
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
TDI	total daily intake
UTR	untranslated region

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REQUESTOR

Competent Authority of The Netherlands

QUESTION NUMBER

EFSA-Q-2023-00051

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX A

Additional studies

List of additional studies performed by or on behalf of the applicant with regard to the evaluation of the safety of maize DP51291 for humans, animals or the environment.

Study identification	Title
PHI-2022-002	Nutritional Equivalency Study of Maize Grain DP-Ø51291-2 – Poultry Feeding Study
PHI-2022-140	Evaluation of Germination and Viability of a Maize Line Containing Event DP-Ø51291-2

APPENDIX B

List of relevant publications identified by the applicant through literature searches (January 2012–September 2024)

Reference

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APPENDIX C

Statistical analysis and statistically significant findings in the 90-day toxicity study in rats

C.1 | Statistical analysis of the 90-day study on maize DP51291 in rats

The following endpoints were statistically analysed: body weights, body weight changes, food consumption, clinical pathology values (as applicable), absolute and relative organ weights, functional observational battery (FOB) data, locomotor activity and histopathological data. For all continuous endpoints, mean, standard deviation in terms of the standardised effect sizes (SES) of each dose group for each sex, variable, and period or time interval were reported. The main statistical analysis compared rats consuming the test diets (at low and high dose) with those consuming the control diet. The statistical model (i.e. at cage, rats or sex specific level) and the experimental unit used, depend on the type of endpoints analysed. Continuous endpoints were analysed with a linear model (factor: treatment, sex and their interaction); for endpoints measured on a discrete scale, the comparisons were performed with Generalised Cochran–Mantel–Haenszel (CMH) or Logistic Regression test. For all other ordinal and nominal (binary) endpoints, no statistical analyses were conducted since all observed values were uniform. Missing data were considered by the Panel and found not to have an impact on the results (Table C.1).

TABLE C.1 Statistically significant findings in 90-day study on maize DP51291 in rats.

Statistically significant parameter/endpoint	Finding	GMO panel interpretation
Body weight, body weight gain and food utilisation	Sporadic changes, increases and decreases during the study (< 10%[a])	Small magnitude. Within normal variation. No impact on terminal body weights or weight gain over the entire study. Not an adverse effect of treatment
FoB, motor activity count	Decreased (10%) in both female groups (interval 1) and in low dose females (20%, interval 3)	Within normal variation, no changes in overall counts. Not an adverse effect of treatment
RBC count	Decreased (5%) in low dose, both groups combined and in males alone	Low magnitude. Not seen in the high dose group. Not an adverse effect of treatment
Haematocrit	Decreased (3%) in low dose, both groups combined and in top dose males	Low magnitude. Within normal variation. Not an adverse effect of treatment
Basophil count	Increased (35%) in top dose males	Within normal variation. No change versus generic diet values
BUN	Decrease (8%) in top dose males	Low magnitude. Not adverse in isolation. Within normal variation. Not an adverse effect of treatment
Glucose	Increased in top dose males (10%) and both sexes combined (8%)	Low magnitude. Within normal variation. Not an adverse effect of treatment
Globulin	Increased in males, (both groups) (5%)	Low magnitude. Within normal variation. Not an adverse effect of treatment
Calcium	Increased (2%–4%) in both female groups, top dose males and both top dose groups combined	Low magnitude. Within normal variation. Not an adverse effect of treatment
TSH	Decreased (10%–12%) in low dose females and both sexes combined	Low magnitude. Not seen at the top dose. Within normal variation, all values within concurrent control range. No associated changes in T3/T4 or thyroid histopathology. Not an adverse effect of treatment
Liver weight (absolute, relative to body wt and brain wt)	Decreased (8%) in low dose females and both sexes combined	Low magnitude. Not seen at the top dose. Within normal variation. No histopathology changes. Not an adverse effect of treatment
Pituitary weight (absolute, relative to brain wt)	Decreased (12%) in low dose females	Low magnitude. Not seen at the top dose. Within normal variation. No histopathology changes. Not an adverse effect of treatment
Thyroid/parathyroid (absolute, relative to brain wt)	Decreased (12%–14%) in both female groups	Low magnitude. Within normal variation. No histopathology changes. Not an adverse effect of treatment

Note: Where changes are given as percentages (e.g. reduced (30%)) this indicates the magnitude of the change relative to the control value (e.g. 30% decrease in mean body weights means a value of 70 g in test group animals vs. 100 g in controls).

[a]Where changes are given as percentages (e.g. reduced (30%)) this indicates the magnitude of the change relative to the control value (e.g. 30% means a value of 7 in test group animals vs. 10 in controls).

APPENDIX D

Animal dietary exposure

TABLE D.1 Dietary exposure to IPD072Aa protein (mg/kg bw per day) in livestock, based on the consumption of maize grain and forage.

	BW (kg)	TDI feed (kg DM/animal)	IR (%)				
			Grain (G)	Forage (F)	G	F	G+F
Broiler	1.7	0.12	70	NA	0.19	–	–
Layer	1.9	0.13	70	10	0.18	0.34	0.52
Turkey	7	0.50	50	NA	0.14	–	–
Breeding pigs	260	6	70	20	0.061	0.23	0.29
Finishing pigs	100	3	70	NA	0.080	–	–
Beef cattle[a]	500	12	80	80	0.073	0.94	1.0
Dairy cattle	650	25	30	60	0.044	1.1	1.2
Ram/ewe	75	2.5	30	NA	0.038	–	–
Lamb	40	1.7	30	30	0.048	0.62	0.67

Note: NA indicates that a forage inclusion rate was not provided in the reference and therefore no exposure calculations were done.
Abbreviations: bw, body weight; TDI, total daily intake.
[a]The inclusion rate for beef cattle would be 160% of the diet, resulting the DDE to each protein an overestimation.

TABLE D.2 Dietary exposure to PAT protein (mg/kg bw per day) in livestock, based on the consumption of maize grain and forage.

	BW (kg)	TDI feed (kg DM/animal)	IR (%)				
			Grain (G)	Forage (F)	G	F	G+F
Broiler	1.7	0.12	70	NA	0.42	–	–
Layer	1.9	0.13	70	10	0.40	0.18	0.59
Turkey	7	0.50	50	NA	0.30	–	–
Breeding pigs	260	6	70	20	0.14	0.12	0.26
Finishing pigs	100	3	70	NA	0.18	–	–
Beef cattle[a]	500	12	80	80	0.16	0.52	0.68
Dairy cattle	650	25	30	60	0.097	0.62	0.72
Ram/ewe	75	2.5	30	NA	0.084	–	–
Lamb	40	1.7	30	30	0.11	0.34	0.45

Note: NA indicates that a forage inclusion rate was not provided in the reference and therefore no exposure calculations were done.
Abbreviations: bw, body weight; TDI, total daily intake.
[a]The inclusion rate for beef cattle would be 160% of the diet, resulting the DDE to each protein an overestimation.

TABLE D.3 Dietary exposure to PMI protein (mg/kg bw per day) in livestock, based on the consumption of maize grain and forage.

	BW (kg)	TDI feed (kg DM/animal)	IR (%)				
			Grain (G)	Forage (F)	G	F	G+F
Broiler	1.7	0.12	70	NA	0.35	–	–
Layer	1.9	0.13	70	10	0.34	0.12	0.46
Turkey	7	0.50	50	NA	0.25	–	–
Breeding pigs	260	6	70	20	0.11	0.083	0.20
Finishing pigs	100	3	70	NA	0.15	–	–
Beef cattle[a]	500	12	80	80	0.14	0.35	0.48
Dairy cattle	650	25	30	60	0.082	0.42	0.50
Ram/ewe	75	2.5	30	NA	0.071	–	–
Lamb	40	1.7	30	30	0.091	0.23	0.32

Note: NA indicates that a forage inclusion rate was not provided in the reference and therefore no exposure calculations were done.
Abbreviations: bw, body weight; TDI, total daily intake.
[a]The inclusion rate for beef cattle would be 160% of the diet, resulting the DDE to each protein an overestimation.