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SCIENTIFIC OPINION

Assessment of genetically modified maize DP915635 for food and feed uses, under regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2020-172)

EFSA Panel on Genetically Modified Organisms (GMO) | Ewen Mullins | Jean-Louis Bresson | Tamas Dalmay | Ian Crawford Dewhurst | Michelle M. Epstein | Leslie George Firbank | Philippe Guerche | Jan Hejatko | Francisco Javier Moreno | Hanspeter Naegeli | Fabien Nogue | Nils Rostoks | Jose Juan Sánchez Serrano | Giovanni Savoini | Eve Veromann | Fabio Veronesi | Michele Ardizzone | Ana M. Camargo | Giacomo De Sanctis | Silvia Federici | Antonio Fernandez Dumont | Andrea Gennaro | Jose Angel Gomez Ruiz | Tilemachos Goumperis | Dafni Maria Kagkli | Paolo Lenzi | Aleksandra Lewandowska | Franco Maria Neri | Nikoletta Papadopoulou | Tommaso Raffaello

Correspondence: nif@efsa.europa.eu

Abstract

Genetically modified maize DP915635 was developed to confer tolerance to glufosinate herbicide and resistance to corn rootworm pests. These properties were achieved by introducing the *ipd079Ea, mo-pat* and *pmi* 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 DP915635 and its conventional counterpart needs further assessment, except for the levels of crude protein in forage, which does not raise nutritional and safety concerns. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the IPD079Ea, PAT and PMI proteins expressed in maize DP915635. The GMO Panel finds no evidence that the genetic modification impacts the overall safety of maize DP915635. In the context of this application, the consumption of food and feed from maize DP915635 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that maize DP915635 is as safe as the 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 DP915635 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 DP915635. The GMO Panel concludes that maize DP915635 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

DP915635, genetic engineering, GM, import and processing, IPD079Ea, maize (*Zea mays*), PAT, PMI

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SUMMARY

Following the submission of application EFSA-GMO-NL-2020-172 under Regulation (EC) No 1829/2003 from Pioneer Hi-Bred International, Inc. as represented by Pioneer Overseas Corporation (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) insect resistant maize (*Zea mays* L.) DP915635 (Unique Identifier DP-915635-4) according to Regulation (EU) No 503/2013. The scope of application EFSA-GMO-NL-2020-172 is for import, processing, and food and feed uses within the European Union (EU) of maize DP915635 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 DP915635 according to the scope of the application EFSA-GMO-NL-2020-172. The GMO Panel conducted the assessment of maize DP915635 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 DP915635 contains a single insert consisting of one copy of the *ipd079Ea, mo-pat*, and *pmi* expression cassettes. Updated 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 IPD079Ea, PAT and PMI proteins is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of plant- and microbe-produced IPD079Ea protein, indicate that these proteins are equivalent and the microbial-derived proteins can be used in the safety studies.

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 DP915635 and its conventional counterpart needs further assessment, except for the levels of crude protein in forage, which does not raise nutritional and safety concerns. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the IPD079Ea, PAT and PMI proteins as expressed in maize DP915635. The GMO Panel finds no evidence that the genetic modification impacts the overall safety of maize DP915635. In the context of this application, the consumption of food and feed from maize DP915635 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that maize DP915635 is as safe as the 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 DP915635 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 DP915635.

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 issues pertaining to the intended uses of maize DP915635.

The GMO Panel concludes that maize DP915635 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 EFSA-GMO-NL-2020-172 is for food and feed uses, import and processing of maize DP915635 and does not include cultivation in the European Union (EU). Maize DP915635 was developed to confer tolerance to glufosinate herbicide and resistance to corn rootworm pests.

1.1 | Background and Terms of Reference as provided by the requestor

On 15 December 2020, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands application EFSA-GMO-NL-2020-172 for authorisation of maize DP915635 (Unique Identifier DP-915635-4), submitted by Pioneer Hi-Bred International, Inc. as represented by Pioneer Overseas Corporation (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003.[1] Following receipt of application EFSA-GMO-NL-2020-172, EFSA informed EU Member States and the European Commission, and made the application available to them. Simultaneously, EFSA published summary of the application.[2]

[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/study-inventory/EFSA-Q-2020-00834.

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 11 June 2021, EFSA declared the application valid.

From validity date, EFSA and its scientific Panel on Genetically Modified Organisms (hereafter referred to as 'the GMO Panel') endeavoured to respect a time limit of 6 months to issue a scientific opinion on application EFSA-GMO-NL-2020-172. 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 'Documentation', below). In accordance with Regulation (EC) No 1829/2003, EFSA consulted the nominated risk assessment bodies of EU Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC.[4] The EU Member States had 3 months to make their opinion known on application EFSA-GMO-NL-2020-172 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 DP915635 in the context of its scope as defined in application EFSA-GMO-NL-2020-172. According to Regulation (EC) No 1829/2003, this scientific opinion is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation and thus will be part of the EFSA overall opinion in accordance with Articles 6(5) and 18(5). 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.[5]

2 | DATA AND METHODOLOGIES

2.1 | Data

The GMO Panel based its scientific risk assessment of maize DP915635 on the valid application EFSA-GMO-NL-2020-172, additional information provided by the applicant during the risk assessment, relevant scientific comments submitted by the Member States and relevant peer-reviewed scientific publications.

2.2 | Methodologies

The GMO Panel conducted its assessment in line with the principles described in Regulation (EU) No 503/2013, the applicable guidelines (i.e. EFSA GMO Panel, 2010a, 2011a, 2011b, 2015a, 2017; EFSA Scientific Committee, 2011) and explanatory notes and statements (i.e. EFSA, 2010, 2014, 2017, 2018, 2019a, 2019b; EFSA GMO Panel, 2010b, 2018, 2021) for the risk assessment of GM plants.

For this application, in the context of the contracts: OC/EFSA/GMO/2020/01, OC/EFSA/GMO/2018/04, OC/EFSA/GMO/2018/02, and EOI/EFSA/SCIENCE/2020/01–CT02GMO, the contractors performed preparatory work for the evaluation of the applicant's literature search, methods applied for the statistical analysis, completeness and quality of DNA sequencing information and statistical analysis of the 90-day toxicity study on maize DP915635, respectively.

3 | ASSESSMENT

3.1 | Introduction

DP915635 maize was genetically modified to confer tolerance to glufosinate herbicide and resistance to corn rootworm pests. Maize DP915635 expresses the IPD079Ea protein for control of corn rootworm pests, phosphinothricin acetyltransferase (PAT) protein that confers tolerance to the glufosinate ammonium-containing herbicides, 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]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]These particulars are available online at: https://open.efsa.europa.eu/study-inventory/EFSA-Q-2020-00834.

3.2 | Systematic literature review as requested by Commission Regulation (EU) No 503/2013[6]

The GMO Panel assessed the applicant's literature searches on maize DP915635, including a scoping review, according to the guidelines given in EFSA (2010, 2019b).

A systematic review referred to in Regulation (EU) No 503/2013 has not been provided to support the risk assessment of application EFSA-GMO-NL-2020-172. Based on the outcome of the scoping review, the GMO Panel agrees that there is limited value of undertaking a systematic review for maize DP915635 at present.

The GMO Panel considered the overall quality of the performed literature searches acceptable. The literature searches identified five relevant peer-reviewed publications on maize DP915635 (Appendix B). Based on the relevant publications,[7] the GMO Panel does not identify any safety issues pertaining to the intended uses of maize DP915635.

3.3 | Molecular characterisation[8]

3.3.1 | Transformation process and vector constructs

Maize DP915635 was developed by site-specific integration (SSI) using two sequential transformation steps:

1. Microprojectile co-bombardment and a CRISPR-Cas9-mediated targeted insertion process to insert a 'landing pad' sequence, at a specific location of the maize genome (inbred PHR03 line), using four plasmids (PHP73878, PHP70605, PHP21139 and PHP21875).
2. *Agrobacterium tumefaciens* (also known as *Rhizobium 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 LBA4404 containing the vector PHP83175.

After the first transformation step, the expression of the zm45CR1 guide RNA leads the Cas9 protein to produce a double-strand break in a targeted location in the maize genome. The break induces a homology-directed repair (HDR) mechanism, allowing a recombination between the zm-SEQ158 and zm-SEQ159 sequences from PHP73878 and the identical endogenous sequences present in the maize genome. As a result of the recombination event, the landing pad introduced in the plant genome the *loxP* site, the maize (*Z. mays*) ubiquitin (*ubiZM1*) 5'-UTR intron and promoter and the *nptII* gene with *pinII* terminator flanked by the flippase recombination sites FRT1 and FRT6. To improve regeneration, two more plasmids, PHP21139 and PHP21875 expressed the WUS2 and the ODP2 proteins, respectively. The guide RNA, the *cas9*, the *wus2* and the *odp2* genes were all transiently expressed, without integration in the plant genome.

The plasmid PHP73878, used to insert the landing pad, contains one expression cassette between the right and left border of the T-DNA, containing the following genetic elements:

– The *nptII* 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 nptII gene from *Escherichia coli*, the terminator region from the *pinII* gene of *Solanum tuberosum*. The nptII coding region and the terminator are flanked by the FRT1 and FRT6 recombination sites, intended to facilitate recombination after the second transformation step with plasmid PHP83175. The entire cassette, which contains a loxP site as well, is flanked by the zm-SEQ158 and zm-SEQ159 sequences, homologous to the maize genome and used to drive the insertion of the landing pad by homologous recombination (HR).

The plasmid PHP70605 contains two expression cassettes consisting of the following genetic elements:

– The *cas9* 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 exon 1 and exon 2 of the coding region of the *cas9* gene, interrupted by the LS1 intron of *Solanum tuberosum*. The coding region is flanked by the SV40 and VirD2 nuclear localisation signals (NLS).
– The zm-45CR1 guide RNA cassette consists of the promoter region from the maize U6 polymerase III, the zm-45CR1 guide RNA from *Z. mays*, and the terminator region of the U6 polymerase III from *Z. mays*.

The plasmid PHP21139 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 *wus2* coding sequence of the Wuschel2 (*wus2*) gene of *Z. mays* and the terminator region from the maize *In2-1* gene of *Z. mays*.

[6]Dossier: Part II – section 7; additional information:18/2/2022; 30/6/2022; 30/6/2022; 25/8/2023; 7/11/2023.
[7]The GMO Panel noted that in the updated literature search, the Boeckman et al. (2022) publication describing the spectrum of activity of IPD079Ea protein was retrieved but not extracted as relevant by the applicant. The GMO Panel considered the article relevant but concluded that it does not add new information that would raise concerns for safety.
[8]Dossier: Part II – section 1.2; additional information: 1/9/2021; 5/11/2021; 30/6/2022; 9/9/2022; 31/5/2023.

The plasmid PHP21875 contains one expression cassette, consisting of the following genetic elements:

– 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 *odp2* coding sequence of the ovule development protein 2 (*odp2*) gene of *Z. mays*, and the terminator region from the *pinII* gene of *Solanum tuberosum*.

In all the above-mentioned plasmids, the vector backbones contained elements necessary for the maintenance and selection of the plasmid in bacteria.

In the second step, an *Agrobacterium*-mediated transformation was used to deliver plasmid PHP83175. The T-DNA of plasmid PHP83175 contains a total of six gene cassettes, of which three gene cassettes (*ipd079Ea, mo-pat* and *pmi*) and one *loxP* site, contained between the FRT1 and FRT6 sites are intended for incorporation into the landing pad of DP915635 maize genome by flippase-mediated recombination, replacing the *nptII* cassette introduced with the landing pad in the first step. The remaining three gene cassettes (*zm-wus2, zm-odp2* and *mo-Flp*) are transiently expressed without integration into the maize genome.

The three expression cassettes outside of the FRT1 and FRT6 sites consist of the following genetic elements:

– The *zm-wus2* gene cassette consists of the promoter and intron region of the *Oryza sativa os*-actin gene, the *wus2* coding sequence of the *Wuschel2 (wus2)* gene of *Z. mays* and the terminator region from the proteinase inhibitor II (*pinII*) gene of *Solanum 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 *odp2* coding sequence of the ovule development protein 2 *(odp2)* gene of *Z. mays* and the terminator region from the *pinII* gene of *Solanum 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 *Saccharomyces cerevisiae*, separated by an intron region from the *LS1* (*st-LS1*) gene of *Solanum tuberosum* and the terminator region from the *pinII* gene of *Solanum tuberosum*. An additional terminator is present between the third and fourth cassettes: the 35S terminator region from the cauliflower mosaic virus genome (CaMV 35S terminator).

The three expression cassettes between FRT1 and FRT6 sites that integrated into the maize genome consist of the following genetic elements:

– The *pmi* gene cassette consists of the pmi coding sequence of the phosphomannose isomerase (*pmi*) gene from *E. coli* and the terminator region from the *pinII* gene of *Solanum tuberosum*. An additional terminator is present between the fourth and fifth cassettes: the terminator region from *Z19* gene of *Z. mays*.
– 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 (*mo-pat*) from *Streptomyces viridochromogenes*, and CaMV 35S terminator. Two additional terminators are present between the fifth and sixth cassettes: the terminator regions from the ubiquitin (*sb-ubi*) and γ-kafarin (*sb-gkaf*) genes of *Sorghum bicolor*.
– The *ipd079Ea* gene cassette consists of three copies of the enhancer region, showing root-specific activity, from the root cortical RCc3 (*sb-RCc3*) gene of *Sorghum bicolor*, the promoter region up-stream of a PCO118362 mRNA sequence (*zm-PCOa*) of *Z. mays*, the intron region from the *Z. mays* ortholog of a rice hypothetical protein (*zm-HPLV9*) gene predicted to be a calmodulin 5 gene, the coding sequence of the insecticidal protein gene *ipd079Ea* of *Ophioglossum pendulum* and the terminator region from the subtilisin-chymotrypsin inhibitor 1B (*sb-SCI-1B*) gene of *Sorghum bicolor*. Three additional terminators are present: the terminator region from the 27-kDa gamma zein (*Z27G*) gene of *Z. mays* W64 line, the terminator region from the ubiquitin 14 (*UBQ14*) gene of *Arabidopsis thaliana* and the terminator region from the maize *In2-1* gene of *Z. mays*.

The vector backbone contained 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 DP915635 was performed by Southern-by-sequencing (SbS) and junction sequence analysis (JSA) to determine the insert copy number and to confirm the absence of plasmid backbone sequences, by polymerase chain reaction (PCR) followed by Sanger sequencing to determine the size and organisation of the inserted sequences, and by DNA sequence analysis. The approach used is acceptable in terms of coverage and sensitivity. 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).

NGS/JSA of the whole genome demonstrated that maize DP915635 contains a single insert, consisting of a single copy of the T-DNAs deriving from PHP73878 and PHP83175. NGS/JSA also confirmed the absence of plasmid backbone sequences in the maize genome.

Sanger sequencing of PCR amplified fragments determined the nucleotide sequence of the entire maize DP915635 event consisting of 20,564 bp of the insert together with 2257 bp of the 5′ and 2046 bp of the 3′ flanking regions. The Sanger analysis revealed that the insert in maize DP915635 is identical to the to the intended landing pad sequence from plasmid PHP73878 and the T-DNA sequence from plasmid PHP83175, except for a single nucleotide A to C change at bp 2931 in the ubiZM1 promoter of the landing pad sequence.

The possible interruption of known endogenous maize genes by the insertion in maize DP915635 was evaluated by bioinformatics analyses of the pre-insertion locus and the genomic sequences flanking the insert. The results of these analyses do not indicate the interruption of any known endogenous gene in maize DP915635.

The results of segregation (see Section 3.3.5) and bioinformatics analyses establish that the insert is located in the nuclear genome.

Updated bioinformatics analyses of the amino acid sequence of the newly expressed IPD079Ea, PAT and PMI proteins reveal no significant similarities to toxins and allergens. In addition, updated bioinformatic analyses of the newly created open reading frames (ORFs) within the insert and spanning the junctions between the insert and genomic DNA indicated that 6 ORFs (DP915635_224, DP915635_498, DP915635_844, DP915635_1097, DP915635_1098, DP915635_1258) exceeded the allergenicity assessment threshold of 35% identity using an 80 amino acid sliding window approach. ORF DP915635_498 is found within the transcriptional unit of the IPD079Ea coding sequence, but in a different reading frame and contain a start codon only close to the C-terminus. ORFs DP915635_224 spans the PAT coding sequence, starting in the actin intron and ending in the CaMV 35S terminator but contains a start codon only close to the C-terminus. DP915635_844, DP915635_1097, DP915635_1098, DP915635_1258 are all in the complementary strand and lack promoter elements. In conclusion, these analyses indicated that the expression of any ORF showing significant similarities to toxins or allergens in maize DP915635 is unlikely.

In order to assess the possibility for horizontal gene transfer (HGT) by HR, the applicant performed a sequence identity analysis for maize DP915635 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 DP915635 expresses three proteins: the IPD079Ea protein for control of corn rootworm pests, the PAT protein which is a phosphinothricin acetyltransferase enzyme that confers tolerance to the glufosinate ammonium-containing herbicides and the PMI protein that was used as a selectable marker. Given the technical restraints in producing large enough quantities from plants, IPD079Ea was recombinantly produced in *E. coli*. A set of biochemical methods was employed to demonstrate the equivalence between the maize DP915635 and *E. coli*-produced IPD079Ea. Purified proteins from these two sources were characterised and compared in terms of their biochemical, structural and functional properties. No PMI or PAT were recombinantly produced in a heterologous expression system and therefore no equivalence analysis was carried out for these two proteins. However, a similar set of biochemical methods was employed to characterise these proteins as produced in maize DP915635.

IPD079Ea protein characterisation and equivalence

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and western blot analyses showed that both plant- and microbe-produced IPD079Ea proteins had the expected molecular weight of ~52 kDa and were comparably immunoreactive to IPD079Ea protein-specific antibodies. Glycosylation detection analysis demonstrated that none of the IPD079Ea proteins were glycosylated. Amino acid sequence of the plant-derived IPD079Ea protein by mass spectrometry (MS) and Edman degradation methods showed that the protein matched the deduced sequence and molecular weight as defined by the *ipd079Ea* gene. In addition, the MS data showed that the N-terminal residue of the protein was acetylated and the N-terminal methionine was absent from both IPD079Ea proteins. Functional equivalence was demonstrated by an *in vitro* assay, which showed that plant- and microbe-derived IPD079Ea proteins had comparable insecticidal activity.

The protein characterisation data comparing the biochemical, structural and functional properties of plant- and microbe-produced IPD079Ea indicate that these proteins are equivalent, and the microbial-derived proteins can be used in the safety studies.

PAT protein characterisation

SDS–PAGE and western blot analyses showed that the 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 protein is not glycosylated. Amino acid sequence analysis of the plant-derived PAT protein by MS and N-terminal sequencing 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 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, 2022a).

PMI protein characterisation

SDS–PAGE and western blot analyses showed that the 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 protein is not glycosylated. Amino acid sequence analysis of the plant-derived PMI protein by MS and N-terminal sequencing showed that the protein matched the deduced sequence as defined by the *pmi* gene.

3.3.4 | Information on the expression of the insert

Protein levels of IPD079Ea, PAT and PMI were analysed by enzyme-linked immunosorbent assay (ELISA) in material harvested in a field trial across five locations in the USA and one location in Canada during the 2019 growing season. Samples analysed included leaves (BBCH 19 and BBCH 85), root (BBCH 16, BBCH 19 and BBCH 85), forage (BBCH 85), grains (BBCH 87–99) and pollen (BBCH 63–65) from plants treated and not treated with glufosinate. The mean values, standard deviations and ranges of protein expression levels in grains ($n = 24$), forage ($n = 24$) and pollen ($n = 24$) of the IPD079Ea, 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, standard deviations and ranges of newly expressed proteins in grains [ng/mg dry weight (dw) and ng/mg fresh weight (fw)], forage and pollen (ng/mg dw) from maize DP915635 ($n = 24$).

	Treatment with glufosinate			
	Not treated		Treated	
Tissues	ng/mg dry weight (dw)	ng/mg fresh weight (fw)	ng/mg dry weight (dw)	ng/mg fresh weight (fw)
Grains (BBCH 87–99)				
IPD079Ea	0.33[a] ± 0.12[b] (0.14–0.65)[c]	0.28 ± 0.10 (0.12–0.55)	0.29 ± 0.12 (< LOQ – 0.55)[d]	0.24 ± 0.10 (< LOQ – 0.46)[d]
PAT	9.3 ± 2.2 (5.7–14)	7.8 ± 1.8 (4.8–12)	11 ± 4.2 (2.3–19)	9.2 ± 3.5 (1.9–16)
PMI	6.0 ± 2.1 (2.9–11)	5.0 ± 1.8 (2.4–9.2)	7.7 ± 3.5 (2.7–16)	6.5 ± 2.9 (2.3–13)
Forage (BBCH 85)				
IPD079Ea	0.32 ± 0.11 (0.11–0.59)		0.31 ± 0.092 (0.15–0.51)	
PAT	16 ± 4.2 (6–23)		15 ± 3.8 (6.7–27)	
PMI	16 ± 3.1 (11–22)		17 ± 2.5 (11–22)	
Pollen (BBCH 63-65)				
IPD079Ea	1.1 ± 0.21 (0.67–1.5)		1.0 ± 0.23 (0.72–1.5)	
PAT	83 ± 14 (49–110)		82 ± 10 (67–100)	
PMI	24 ± 3.0 (18–28)		23 ± 2.6 (18–28)	

[a]Mean value.

[b]Standard deviation.

[c]Range.

[d]IPD079Ea levels in grains were below the limit of quantification (LOQ = 0.069 ng/mg dw and 0.058 ng/mg fw) in two out of 24 treated samples. A value equal to half the LOQ value was assigned to those samples to calculate the mean and standard deviation.

3.3.5 | Inheritance and stability of inserted DNA

Genetic stability of the maize DP915635 insert was assessed by Southern blot analysis on five generations (T1, T2, T3, T4 and T5) while the inheritance pattern was assessed by quantitative polymerase chain reaction (qPCR)-based segregation analysis and phenotypic analysis (resistance to glufosinate) from 5 generations (F1, 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 DP915635 contains a single insert consisting of one copy of the *ipd079Ea, mo-pat* and *pmi* expression cassettes deriving from the landing pad sequence from plasmid PHP73878 and the T-DNA sequence from plasmid PHP83175. 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 trait is confirmed over several generations. The methodology used to quantify the levels of the IPD079Ea, PAT and PMI proteins is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of plant- and microbe-produced IPD079Ea proteins, indicate that these proteins are equivalent and the microbial-derived protein can be used in the safety studies.

3.4 | Comparative analysis[9]

3.4.1 | Overview of studies conducted for the comparative analysis

Application EFSA-GMO-NL-2020-172 presents data on agronomic and phenotypic characteristics, as well as on forage and grain composition of maize DP915635 (Table 2). In addition, the application contains data on seed germination characteristics of F_1 grains from maize DP915635 (Appendix A).

TABLE 2 Main comparative analysis studies to characterise maize DP915635 provided in the application EFSA-GMO-NL-2020-172.

Study focus	Study details	Comparator	Non-GM reference varieties
Agronomic and phenotypic analysis	Field study, USA and Canada, 2019, 11 sites[a,b]	PH1KFT/PHR03	20[c]
Compositional analysis	Field study, USA and Canada, 2019, eight sites[a]		

Abbreviation: GM, genetically modified.

[a]Two of the field trials in USA were located in Illinois, one in Iowa-1 Indiana, Nebraska, Pennsylvania and Texas and the field trial in Canada was located in Ontario.

[b]Three additional field trials located in Ontario (Canada), Iowa-2 and Texas (USA) were used only for the agronomic and phenotypic analysis. An additional site present in Missouri was excluded, due to absence of data collected from this site.

[c]Non-GM maize hybrid with their corresponding comparative relative maturity indicated in brackets were 5513 (105), P0506 (105), 35A52 (107), P0604 (106), P0760 (107), 5883 (108), P0993 (109), 5939 (109), 5828 (110), P1151 (111), P1197 (111), 6158 (111), P0928 (109), P1105 (111), P1345 (113), P1319 (113), P1395 (113), P1422 (114), 33Y74 (115), 6575 (115).

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 DP915635 not exposed to the intended herbicide, maize DP915635 exposed to the intended herbicide, the comparator PH1KFT/PHR03 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 DP915635, 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).[10]

3.4.3 | Suitability of selected test materials

3.4.3.1 | *Selection of the test materials*

As described in Section 3.3.1 inbred line PHR03 was transformed to obtain maize DP915635, which was then crossed with the inbred line PH1KFT to produce the hybrid maize DP915635 PH1KFT/PHR03 used in the comparative analysis.

The comparator used in the field trials is the non-GM maize hybrid PH1KFT/PHR03, which has a similar genetic background as maize DP915635 (as documented by the pedigree), and is therefore considered to be the conventional counterpart.

Maize DP915635 and the conventional counterpart (PH1KFT/PHR03), both with a comparative relative maturity (CRM) of 110, are appropriate for growing in environments across North America, where the comparative field trials were conducted.

Commercial non-GM reference varieties with a CRM ranging from 105 to 115 were selected by the applicant and, at each selected site, four reference varieties were tested (see Table 1). On the basis of the provided information on relative maturity classes, the GMO Panel considers the selected non-GM reference varieties appropriate for the comparative assessment.

[9]Dossier: Part II – section 1.3; additional information: 1/9/2021, 5/11/2021, 18/2/2022.

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

3.4.3.2 | *Seed production and quality*

Seeds of maize DP915635 and the conventional counterpart used in the 2019 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 quantitative polymerase chain reaction analysis.

The grains were tested for their germination capacity under warm and cold temperature conditions.[11] Maize DP915635 and its comparator were compared for germination capacity and the results[12] of these studies indicate that the seed germination of maize DP915635 was not different than that of its comparator.

3.4.3.3 | *Conclusion on suitability*

The GMO Panel is of the opinion that the maize DP915635, the 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 trials sites were located in commercial maize-growing regions of the USA and Canada. The soil and climatic characteristics of the selected fields were diverse,[13] 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 five of the selected sites.[14] Due to the lack of major impacts on plant growth at the other sites, the GMO Panel considers that the exceptional weather conditions did not invalidate the selection of the field trial sites for the comparative analysis. In addition, some extreme weather events were recorded at one of the selected sites.[15] As the quality of the field trial site was compromised, only the endpoints collected before the occurrence of the extreme weather events were included in the statistical analysis.

3.4.4.3 | *Management practices*

The field trials included plots containing maize DP915635, plots with the conventional counterpart and plots with non-GM maize reference varieties, mostly managed according to local agricultural practices. In addition, the field trials included plots containing maize DP915635 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 stages.[16] At some field trial sites,[17] sowing occurred later than usual, resulting in a shorter growing cycle. The additional information indicated that the shorter growing cycle was unlikely to affect the representativeness of field trial conditions. The GMO Panel considers that the management practices, including sowing, harvesting and application of plant protection products were acceptable for the selected receiving environments.

3.4.4.4 | *Conclusion on representativeness*

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

3.4.5 | Agronomic and phenotypic analysis

Eleven agronomic and phenotypic endpoints[18] plus information on abiotic stressors, disease incidence and arthropod damage were collected from the field trials sites (see Table 1). The endpoints lodging, ear count and dropped ears were not analysed with formal statistical methods because of lack of variability in the data.

[11]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.

[12]GM hybrid maize showed a mean germination of 97% and 95% while the non-GM comparator showed a mean of 96% and 95% under warm and cold temperature conditions, respectively.

[13]Soil types of the field trials were clay, clay loam, silty clay loam, silt loam, loam and sandy loam; soil organic matter ranged from 0.5% to 2.8%; pH ranged from 5.8 to 8.2; average temperatures and sum of precipitations during the usual crop growing season ranged respectively from 14.6 to 27.9°C and from 351 to 626 mm.

[14]Hail and windstorm were registered at one field trial located in Iowa (Iowa-1), and windstorm in Texas, heavy rainfall in Pennsylvania and Texas and frost damage in Ontario (Canada).

[15]One field trial established in Iowa (Iowa-2) was compromised by hail and windstorm occurred at the beginning of the crop growth season, thus only early stand count was included in the statistical analysis.

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

[17]Two field trials located in Nebraska and Texas.

[18]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.

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

- For maize DP915635 (not treated with the intended herbicide), the test of difference identified statistically significant differences with the comparator for early stand count and days to flowering. Both endpoints fell under equivalence category I.
- For maize DP915635 (treated with the intended herbicide), the test of difference identified no statistically significant differences with the comparator.

3.4.6 | Compositional analysis

Forage and grain of maize DP915635 harvested from the field trials (Table 2) were analysed for 80 constituents (10 in forage and 70 in grain), including those recommended by OECD (2002). The statistical analysis as described in Section 3.4.2 was not applied to 10 grain constituents[19] because their concentrations in more than half of the samples were below the limit of quantification.

The statistical analysis was applied to a total of 70 constituents (10 in forage[20] and 60 in grain[21]); a summary of the outcome of the test of difference and the test of equivalence is presented in Table 3:

- For maize DP915635 not treated with the intended herbicides, statistically significant differences with the non-GM comparator were found for eight endpoints (all in grains). All these endpoints fell under equivalence category I or II.
- For maize DP915635 treated with the intended herbicides, statistically significant differences with the non-GM comparator were found for 11 endpoints (1 in forage and 10 in grains). All these endpoints fell under equivalence category I or II except for crude protein in forage, which fell under equivalence category III.

TABLE 3 Outcome of the comparative compositional analysis of forage and grain of maize DP915635. The table shows the number of endpoints in each category.

		Test of difference[a]			
		Not treated[c]		Treated[c]	
		Not different	Significantly different	Not different	Significantly different
Test of equivalence[b]	Category I/II	62	8[d]	58	10[d]
	Category III/IV	–	–	1[e]	1[f]
	Not categorised	–	–	–	–
	Total endpoints	70		70	

[a]Comparison between maize DP915635 and its comparator.

[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 herbicides.

[d]Endpoints with significant differences between maize DP915635 and its comparator and falling under equivalence category I–II. For forage, none. For grains, not treated only: oleic acid (C18:1), linoleic acid (C18:2), eicosenoic acid (C20:1), pyridoxine; treated only: NDF, calcium, iron, pantothenic acid, p-coumaric acid, inositol; both treated and not treated: palmitoleic acid (C16:1), stearic acid (C18:0), lignoceric acid (C24:0), methionine.

[e]Endpoint with no significant differences between maize DP915635 and its comparator and falling under equivalence category III: carbohydrates (in forage, treated only).

[f]Endpoint with significant differences between maize DP915635 and its comparator and falling in equivalence category III/IV: crude protein (in forage, treated only). Quantitative results for this endpoint are reported in Table 7.

The GMO Panel assessed all the significant differences between maize DP915635 and its comparator, taking into account the potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties. Quantitative results for the endpoint showing significant differences between maize DP915635 and its comparator and falling under equivalence category III/IV are given in Table 4.

[19]Lauric acid (C12:0), myristic acid (C14:0), heptadecenoic acid (C17:1), eicosadienoic acid (C20:2), copper, riboflavin, β-tocopherol, δ-tocopherol, furfural and raffinose.

[20]Moisture, crude protein, crude fat, ash, carbohydrates, crude fibre, acid detergent fibre (ADF), neutral detergent fibre (NDF), calcium and phosphorus.

[21]Proximates and fibre content (ash, carbohydrates, crude fat, crude fibre, crude protein, moisture, acid detergent fibre (ADF), neutral detergent fibre (NDF) and total dietary fibre (TDF)), minerals (calcium, iron, magnesium, manganese, phosphorus, potassium, sodium, zinc), vitamins (α-tocopherol, β-carotene, γ-tocopherol, total tocopherols, thiamine, niacin, pantothenic acid, pyridoxine, 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), heptadecanoic acid (C17:0), 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), behenic acid (C22:0), lignoceric acid (C24:0)) and other compounds (ferulic acid, inositol, *p*-coumaric acid, phytic acid, trypsin inhibitor).

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

		Maize DP915635			Non-GM reference varieties	
	Endpoint	Not treated[a]	Treated[a]	Comparator	Mean	Equivalence limits
Forage	Crude protein (% dw)	7.13	6.85*	7.23	7.72	7.00–8.44

Note: For maize DP915635, 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) and light grey (equivalence category III).

Abbreviation: dw, dry weight.

[a]Treated: treated with the intended herbicide; not treated: treated only with conventional herbicides (see Section 3.4.4.3).

3.4.7 | Conclusions on the 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 were 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 the agronomic and phenotypic characteristics between maize DP915635 and the comparator needs further assessment for environmental safety.
- None of the differences identified in forage and grain composition between maize DP915635 and the comparator needs further assessment regarding food and feed safety except for the levels of crude protein in forage (treated), which is further assessed in Section 3.4.3.

3.5 | Food/feed safety assessment[22]

3.5.1 | Effects of processing

Maize DP915635 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 | Stability of newly expressed proteins

Protein stability is one of several relevant parameters to consider in the weight-of-evidence approach in protein safety assessment (EFSA GMO Panel, 2010c, 2011a, 2017, 2021). The term protein stability encompasses several properties such as thermal stability, pH-dependent stability, proteolytic stability and physical stability (e.g. tendency to aggregate), among others (Li et al., 2019). One of the most prominent traits attributed to food allergens is protein stability (Breiteneder & Mills, 2005; Costa et al., 2022; Foo & Mueller, 2021; Helm, 2001).

3.5.2.1 | *Effect of temperature and pH on newly expressed proteins*

The effects of temperature and pH on PAT and PMI proteins expressed in maize DP915635 were previously evaluated by the GMO Panel (EFSA GMO Panel, 2012, 2015b, 2020). The applicant provided studies on the IPD079Ea protein expressed in maize DP915635. IPD079Ea protein samples were incubated for ~30 min at 25, 50, 75 and 95°C followed by a functional activity bioassay. The studies showed that the IPD079Ea protein was inactive after incubation at temperatures ≥ 50°C. In relation to the effect of pH on the IPD079Ea protein, at pH 1.2 the molecular mass (~52 kDa) of the protein was unchanged.

3.5.2.2 | *In vitro protein degradation by proteolytic enzymes*

In vitro protein degradation studies on PAT and PMI proteins as expressed in maize DP915635 were previously evaluated by the GMO Panel (EFSA GMO Panel, 2012, 2015b, 2020).

Furthermore, the applicant provided information on *in vitro* protein degradation (resistance to pepsin in solutions at pH ~1.2) of the IPD079Ea protein from a microbial recombinant system. The integrity of the test protein in samples of the incubation mixture taken at various time points was analysed by SDS–PAGE followed by protein staining or by western blotting. The IPD079Ea protein was degraded by pepsin within 0.5 min of incubation. Transient peptide fragments of low molecular weight were observed at different time points by SDS–PAGE. Furthermore, the applicant provided a

[22]Dossier: Part II – sections 1.4, 1.5, 1.6, 2, 4; additional information: 1/9/2021, 5/11/2021, 21/12/2021, 18/2/2022, 16/6/2022, 9/9/2022.

study, where the IPD079Ea protein was subjected to a sequential digestion, pepsin followed by pancreatin. The transient peptide fragments seen in the pepsin analysis were degraded within 0.5 min of exposure to pancreatin when analysed by SDS–PAGE. The sequential addition of digestive enzymes – gastric digestion conditions followed by an intestinal *in vitro* digestion – has been proposed as part of several alternative protocols to the classical pepsin resistance test to more closely simulate (within the inherent limitations of *in vitro* models) the physiological conditions of gastrointestinal digestion (EFSA GMO Panel, 2021). This is in line with Codex Alimentarius which indicated that alternative *in vitro* digestion protocols may be used, where adequate justification is provided (Codex Alimentarius, 2009).

3.5.3 | Toxicology

3.5.3.1 | *Testing of newly expressed proteins*

Three proteins (PAT, PMI and IPD079Ea) are newly expressed in maize DP915635. On the basis of the known biological function of the individual newly expressed proteins, there is currently no expectation for their possible interactions relevant to the food and feed safety of maize DP915635.

NEP previously assessed

The PAT and PMI proteins were previously assessed by the GMO Panel in the context of other applications and no safety concerns for humans and animals (i.e. farmed and companion animals) were identified (EFSA GMO Panel, 2012, 2015b, 2020). These proteins have been extensively characterised (see MC section). Updated bioinformatics analyses revealed no similarities of the PAT and PMI proteins with known toxins. The GMO Panel has not identified any new information that would change the previous conclusion on the safety of the PAT and PMI proteins.

NEP never assessed before

The IPD079Ea protein was never assessed by the GMO Panel in the context of its previous opinions, for the safety of humans and animals.

The GMO Panel assessed the safety profile of IPD079Ea protein in maize DP915635, taking into account molecular characterisation and bioinformatic analyses (MC section), the history of safe use for consumption of the newly expressed proteins, and *in vitro* (Section 3.4.2) and *in vivo* studies.

Safety profile of IPD079Ea protein

(i) Molecular characterisation

The plant-produced IPD079Ea protein has been extensively characterised and its equivalence to the microbially produced protein was demonstrated (Section 3.3.3).

(ii) Bioinformatic studies

No significant similarities of the IPD079Ea protein to toxins were identified (see Section MC).

(iii) History of safe use for consumption as food/feed of the newly expressed proteins

a. *Information on the source organism*

The IPD079Ea protein's gene source organism is a fern (*Ophioglossum pendulum*) and the GMO Panel is not aware of any use of this fern as food and feed in Europe on the basis of the available information.

b. *Information on structure, function and mode of action of the new protein*

The IPD079Ea protein is intended to target and disrupt midgut epithelial cells in certain coleopteran species (Western Corn Rootworm, WCR). Once ingested it binds to specific receptors of the midgut enterocytes on their brush border. This is followed by enterocytes morphological degenerative changes, breakdown of the midgut epithelial lining and larvae death. The applicant indicates that receptor binding occurs in acidic conditions, which explains the specificity against WCR among insects.

c. *Overall conclusion on the history of safe use*

The GMO Panel considers the above information not sufficient to duly document the history of safe use for consumption of the newly expressed protein.

(iv) *In vitro* studies

The outcome of *in vitro* studies to characterise the stability of newly expressed proteins has been described in Section 3.5.2.

(v) *In vivo* studies

The outcome of an acute toxicity study and of a 28-day study with the *E. coli*-produced IPD079Ea protein is described below.

Acute toxicity study

An acute toxicity study in CD-1 mice, administrated the *E. coli*-produced IPD079Ea protein by gavage at the dose of 5000 mg/kg body weight (bw) showed no adverse effects.

28-Day repeated dose toxicity study

The applicant provided a 28-day repeated dose toxicity study in mice with the IPD079Ea protein, conducted in accordance with OECD TG 407 (2008) and to the principles of Good Laboratory Practice (GLP).

Groups of Crl:CD-1 mice (10/sex per group), 7–8 weeks old at the start of dosing were allocated to five groups. Groups were administered diets containing, respectively, the test substance (IPD079Ea protein) at targeted nominal doses of 1000, 300 or 100 mg/kg bw per day (high, medium and low IPD079Ea protein groups); 1000 mg/kg bw per day of bovine serum albumin (BSA) (BSA control group) or a basal diet (control group). An additional 10 mice/sex per group were used to investigate coagulation parameters (satellite animals).

The test substance used in this study was produced by a recombinant system and contained about 82% IPD079Ea protein. The amino acid sequence analysis of the *E. coli*-produced IPD079Ea used in this 28-day toxicity study by mass spectrometry matched the deduced sequence as defined by the *DP915635* gene. This protein had the expected molecular weight and immunoreactivity to IPD079Ea-specific antibodies, was not glycosylated and showed functional activity.

In-life procedures and observations and terminal procedures were conducted in accordance with OECD TG 407 (2008), except for satellite animals that were not subjected to some in life procedure (ophthalmology, functional observational battery, motor activity), clinical chemistry and pathology investigations.

The GMO Panel noted that animals were singly housed, and considered the justification provided by the applicant acceptable.[23] Deviations to the protocol reported in the study were considered minor deviations with no impact on the study results.

Based on the results of concentration analysis by ELISA, the applicant confirmed the expected dietary concentrations (0.65, 1.95, 6.5 g/kg diet). The results of the test diet analysis indicated that the diets preparations were homogeneous and exhibited acceptable stability.

An appropriate range of statistical tests was performed on the results of the study and a detailed description of the methodology and of statistically significant findings identified in mice is reported in Appendix C.1.

There were no test diet-related incidents of mortality or clinical signs. 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[24] for the parameter in mice 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 end-points.
– exhibited no consistency with increasing dose levels.

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

The GMO Panel concludes that no adverse effects were observed in mice in this 28-day toxicity study on *E. coli*-produced IPD079Ea protein, at nominal dietary exposures up to 1000 mg/kg bw per day.

(vi) Conclusion on the toxicological profile of IPD079Ea protein

Based on the above information, the GMO Panel did not identify food and feed safety concerns in humans and animals related to the IPD079Ea protein.

3.5.3.2 | *Testing of new constituents other than newly expressed 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 grain and forage from maize DP915635. Therefore, no further food/feed safety assessment of components other than the newly expressed proteins is required.

[23]Males were individually housed because male mice are often aggressive and not considered social; females were housed individually to avoid compromising data interpretation resulting from animals being treated differently and to obtain accurate individual animal food consumption data for calculation of test substance intake.

[24]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 'adverse' 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).

3.5.3.3 | *Information on altered levels of food and feed constituents*

Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, no altered levels of food and feed constituents have been identified in grain and forage of maize DP915635, except for the levels of crude protein in forage (treated). 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 3.5.6 (animal nutrition).

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

Based on the outcome of the molecular characterisation and comparative analysis assessment, no compositional modifications or indication of possible unintended effects relevant to food and feed safety have been identified for maize DP915635. Therefore, animal feeding studies with food/feed derived from maize DP915635 are not considered necessary by the GMO Panel (EFSA GMO Panel, 2011a). In accordance with Regulation (EU) No 503/2013, the applicant provided a 90-day feeding study in rats receiving diets containing from maize DP915635.

In this study, pair-housed Crl:CD(SD) rats (16/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 DP915635 grains from plants treated with the intended herbicide (glufosinate ammonium-containing herbicides) 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 reference varieties (P1197, 6158, and 6365) (inclusion level 50%).

The study was adapted from OECD test guideline 408 (2018), aligned with EFSA Scientific Committee guidance (EFSA Scientific Committee, 2011) and complied with the principles of 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 verified; however, in accordance with product expiration declared by the diet manufacturer, the constituents of the diets are considered stable for the duration of the treatment. The GMO Panel considered this justification acceptable. Diet preparation procedures and regular evaluations of the mixing methods guaranteed the homogeneity and the proper concentration of the test or control substances in them. The applicant provided information on concentration of the newly expressed proteins in the GM diets, further supporting the homogeneity of the GM formulations.

Event-specific PCR analysis confirmed the presence of the event in both the GM maize and diets and excluded the presence of the event in the respective controls. Both the GM maize and diets were analysed for nutrients, antinutrients and potential contaminants (e.g. selected heavy metals, mycotoxins and pesticides). 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 with OECD TG 408 (2018).

An appropriate range of statistical tests was performed on the results of the study. Detailed description of the methodology and of statistically significant findings identified in rats given a diet containing maize DP915635 is reported in Appendix C.2.

There were no test diet-related incidents of mortality or clinical signs. 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[25] 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 end-points;
– exhibited no consistency with increasing dose levels.

No gross pathology findings related to the administration of the test diet were observed at necropsy, and the microscopic examination 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 no adverse effects were observed in rats in this 90-day toxicity study given diets containing maize DP915635 up to 50% incorporation rate.

3.5.4 | 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;

[25]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 'adverse' 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).

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.

3.5.4.1 | *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 proteins, 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 *ipd079Ea*, *pat* and *pmi* genes originate from *O. pendulum*, *S. viridochromogenes* and *E. coli*, respectively, none of which are considered common allergenic sources. The safety of the PAT and PMI proteins have been previously assessed by the GMO Panel and no safety concerns were identified (EFSA GMO Panel, 2012, 2015b, 2017).

Updated bioinformatic analyses of the amino acid sequences of the IPD079Ea, PAT and PMI proteins, using the criterion of 35% identity in a sliding window of 80 amino acids, revealed no relevant similarities to known allergens. The studies on protein stability of the IPD079Ea, PAT and PMI proteins have been described in Section 3.4.2. In addition, the GMO Panel did not find an indication that the newly expressed proteins IPD079Ea, PAT and PMI at the levels expressed in maize DP915635 might be adjuvants.

Furthermore, the applicant provided information on the safety of the IPD079Ea, PAT and PMI proteins regarding their potential hazard to cause a celiac disease response.[26] For such assessment, the applicant followed the principles described in the EFSA GMO Panel guidance document (EFSA GMO Panel, 2017). The assessment of the IPD079Ea 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 and required further investigations. These partial matches have been previously assessed by the EFSA GMO Panel and no safety concerns were identified (EFSA GMO Panel, 2022a, 2022b). Briefly, based on additional considerations on the position and nature of amino acids flanking the motif, such as the presence of two consecutive prolines and the charge and size of adjacent amino acids (EFSA GMO Panel, 2017), the relevant peptides containing the motif do not raise concern as they fail to mimic gluten sequences. Therefore, no indications of safety concerns were identified by the GMO Panel.

In the context of this application, the GMO Panel considers that there are no indications that the newly expressed IPD079Ea, PAT and/or PMI proteins in maize DP915635 may be allergenic.

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

The GMO Panel regularly reviews the available publications on food allergy to maize products. However, to date, maize is not considered a common allergenic food[27] (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 3.2, 3.3 and 3.4), the GMO Panel identifies no indications of a potentially increased allergenicity of food and feed derived from maize DP915635 with respect to that derived from the conventional counterpart and the non-GM reference varieties tested.

3.5.5 | Dietary exposure assessment to new constituents

In line with Regulation (EU) No 503/2013 the applicant provided dietary exposure estimates to IPD079Ea, PAT, and PMI proteins newly expressed in DP915635 maize. Dietary exposure was estimated based on protein expression levels reported in this application for DP915635 maize treated with the intended herbicide, the current available consumption data and feed practices, the foods and feeds currently available in the market and the described processing conditions.

For the purpose of estimating dietary exposure, the levels of the three proteins newly expressed in DP915635 maize grains, pollen and forage were derived from replicated field trials (four replicates from six locations, $n = 24$) in 2019 in the USA and Canada. Table 1 in Section 3.3.4. shows the protein expression levels used to estimate both human and animal dietary exposure.

3.5.5.1 | *Human dietary exposure*

Chronic and acute dietary exposure to IPD079Ea, PAT and PMI proteins newly expressed in maize DP915635 were provided. The applicant followed the methodology described in the EFSA Statement 'Human dietary exposure assessment to newly expressed protein in GM foods' (EFSA, 2019a) to estimate human dietary exposure in average and high consumers making use of summary statistics of consumption.

Human dietary exposure was estimated across different European countries on different population groups: young population (infants, toddlers, 'other children'), adolescents, adult population (adults, elderly and very elderly) and special

[26]Technical dossier section 1.5, additional information 30/6/2022.

[27]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.

populations (pregnant and lactating women). Since no specific consumption data were available on commodities containing, consisting of or obtained from maize DP915635 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).[28] 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.[29] No losses in the newly expressed proteins during processing were considered, except for certain commodities excluded from the exposure estimations (maize oil, corn starch, corn syrup).

The highest acute dietary exposure (high consumers) was estimated in the age class 'Other children' with exposure estimates of 139.8 µg/kg bw per day, 99.8 µg/kg bw per day and 3.6 µg/kg bw per day for PAT, PMI and IPD079Ea proteins, respectively. The main contributor to the exposure in the dietary survey with the highest estimates was corn grains.

The highest chronic dietary exposure (high consumers) was estimated in the age class 'Infants' with exposure estimates of 52.5 µg/kg bw per day, 37.1 µg/kg bw per day and 1.4 µg/kg bw per day for PAT, PMI and IPD079Ea proteins, respectively. The main contributor to the exposure in the dietary survey with the highest estimates was sweet corn.

Additional dietary exposure to IPD079Ea, PAT, and PMI proteins might occur via the consumption of pollen supplements under the assumption that these supplements contain pollen from maize DP915635. From the expression values reported in pollen in Table 1 (see Section 3.3.4), the concentrations of IPD079Ea, PAT, and PMI proteins in pollen supplements were calculated, assuming a moisture content of approximately 6%. Consumption data on pollen supplements are available for few consumers across seven different European countries.[30] The low number of consumers available adds uncertainty to the exposure estimations which should be interpreted with care, and it prevents from estimating exposure for high consumers of pollen supplements. In average consumers of pollen supplements, the highest acute dietary exposure would range from 0.70 µg/kg bw per day for IPD079Ea to 57.1 µg/kg bw per day for PAT, in the elderly population. Similarly, the highest chronic dietary exposure in average consumers would range from 0.46 µg/kg bw per day for IPD079Ea to 38.1 µg/kg bw per day for PAT, also in the elderly population.

3.5.5.2 | *Animal dietary exposure*

Dietary exposure to IPD079Ea, PAT and PMI proteins in maize DP915635 was estimated across different animal species as below described, 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 DP915635 products was considered.

Mean levels (dry weight) of the newly expressed proteins in grains and forage from maize DP915635 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 IPD079Ea, 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.

3.5.6 | Nutritional assessment of endogenous constituents

The intended traits of the DP915635 maize are herbicide tolerance and insect resistance, with no intention to alter nutritional parameters. However, levels of crude protein in forage (treated) were significantly different from its comparator and showed a lack of equivalence with the set of non-GM reference varieties (Section 3.4.7). The biological relevance of this compound, the role of DP915635 maize forage as contributor to the total intake and the magnitude and direction of the observed change were considered during the animal nutritional assessment.

Forage is an important feed source for herbivores that can utilise it because of the capacity for microbial digestion of cell wall constituents. Forage guarantees the proper function of gastrointestinal tract that is essential for the activity of microbes; moreover, forage alone is able to satisfy nutritional requirements of animals up to a certain level, e.g. low producing dairy cows. Therefore, forage is not provided to animals with the only purpose to fulfil nutritional requirements. Maize forage is fed to animals to provide mainly energy and fibre, while the contribution of the protein is limited. The magnitude of the decrease in crude protein in maize DP915635 forage does not represent a nutritional concern.

[28] https://www.efsa.europa.eu/en/applications/gmo/tools. Data accessed: June 2020.

[29] 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 6.0 µg of PAT per gram of maize bread as compared to the 6.6 µg/g (fresh weight, see Table 1 in Section 3.3.4) reported as mean concentration in the maize grains.

[30] https://www.efsa.europa.eu/en/food-consumption/comprehensive-database. Data accessed: May 2022.

3.5.7 | Post-market monitoring of GM food/feed

Maize DP915635, 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.

3.5.8 | Conclusions on the food/feed safety assessment

The proteins IPD079Ea, PAT, and PMI newly expressed in maize DP915635 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. Similarly, 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 DP915635. The GMO Panel finds no evidence that the genetic modification impacts the overall safety of maize DP915635. Based on the outcome of the comparative assessment and the nutritional assessment, the GMO Panel concludes that the consumption of maize DP915635 does not represent any nutritional concern, in the context of the scope of this application. The GMO Panel concludes that maize DP915635, 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[31]

3.6.1 | Environmental risk assessment

Considering the scope of application EFSA-GMO-NL-2020-172, which excludes cultivation, the environmental risk assessment (ERA) of maize DP915635 mainly takes into account: (1) the exposure of microorganisms to recombinant DNA in the gastrointestinal 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 DP915635 seeds/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 DP915635 will provide a selective advantage to maize plants, except when they are exposed to glufosinate ammonium-containing herbicides or infested by insect pests that are susceptible to the IPD079Ea protein. 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 that it is very unlikely that maize DP915635 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 DP915635 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.

3.6.1.2.1 | *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.

[31]Dossier: Part II – sections 5 and 6.

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).

HR 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 DP915635 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 protein were plant codon-optimised and did not provide sufficient sequence identity to bacterial DNA.

In summary, there is no indication for an increased likelihood of horizontal transfer of DNA from maize DP915635 to bacteria. Given the nature of the recombinant DNA, the GMO Panel identified no safety concern linked to an unlikely but theoretically possible HGT.

Plant-to-plant gene transfer

The potential for occasional feral maize DP915635 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, even if exposed to the intended herbicide.

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

Taking the scope of application EFSA-GMO-NL-2020-172 into account (no cultivation), potential interactions of occasional feral maize DP915635 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*

Given that environmental exposure of non-target organisms to spilled GM material or occasional feral GM maize plants arising from spilled maize DP915635 grains is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed with GM maize, the GMO Panel considers that potential interactions of maize DP915635 with non-target organisms do not raise any environmental safety concern.

3.6.1.5 | *Interactions with abiotic environment and biogeochemical cycles*

Given that environmental exposure to spilled GM material or occasional feral maize DP915635 plants arising from grain import spills is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed with GM maize, the GMO Panel considers that potential interactions 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 DP915635, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for maize DP915635 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 DP915635. 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 DP915635 would differ from conventional maize varieties in its ability to persist under European environmental conditions. Considering the scope of application EFSA-GMO-NL-2020-172, interactions of occasional feral maize DP915635 plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from maize DP915635 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 DP915635 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 DP915635.

4 | CONCLUSIONS

The GMO Panel was asked to carry out a scientific assessment of maize DP915635 for import, processing, and food and feed uses in accordance with Regulation (EC) No 1829/2003.

The molecular characterisation data establish that maize DP915635 contains a single insert consisting of one copy of the *ipd079Ea*, *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 trait is confirmed over several generations. The methodology used to quantify the levels of IPD079Ea, PAT and PMI proteins is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of plant- and microbe-produced IPD079Ea proteins, indicate that these proteins are equivalent and the microbial-derived proteins can be used in the safety studies.

None of the identified differences in the agronomic/phenotypic and compositional characteristics tested between maize DP915635 and its conventional counterpart needs further assessment, except for the levels of crude protein in forage, which does not raise nutritional and safety concerns. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of IPD079Ea, PAT and PMI proteins as expressed in maize DP915635, and finds no evidence that the genetic modification would change the overall allergenicity of maize DP915635. In the context of this application, the consumption of food and feed from maize DP915635 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that maize DP915635 is as safe as the conventional counterpart and non-GM maize reference varieties tested, and no post-market monitoring of food/feed is considered necessary.

In the case of accidental release of viable maize DP915635 seeds 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 DP915635. 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 DP915635.

The GMO Panel concludes that maize DP915635 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

- Application submitted for the authorisation of genetically modified maize DP-915635-4 submitted by Pioneer Overseas Corporation on behalf of PioneerHi-Bred International, Inc. on 15 December 2020 (EFSA Ref. EFSA-GMO-NL-2020-172; EFSA-Q-2020-00834)
- The application was made valid on 11 June 2021
- Additional information (1) was requested on 22 June 2021

- Additional information (1) was received on 1 September 2021
- Additional information (2) was requested on 26 August 2021
- Additional information (2) was received on 5 November 2021 partial; 21 December 2021 full
- Additional information (3) was requested on 16 December 2021
- Additional information (3) was received on 18 February 2022
- Additional information (4) was requested on 24 March 2022
- Additional information (4) was received on 30 June 2022
- Additional information (5) was requested on 30 May 2022
- Additional information (5) was received on 16 June 2022
- Additional information (6) was requested on 14 July 2022 (JRC-EURL)
- Additional information (6) was received on 16 November 2023
- Additional information (7) was requested on 15 July 2022
- Additional information (7) was received on 9 September 2022
- Additional information (8) was requested on 31 March 2023
- Additional information (8) was received on 31 May 2023
- Additional information (9) was requested on 27 June 2023
- Additional information (9) was received on 25 August 2023
- Additional information (10) was requested on 19 October 2023
- Additional information (10) was received on 7 November 2023

ABBREVIATIONS

ADF	acid detergent fibre
bp	base pair
BSA	bovine serum albumin
bw	body weight
CaMV	cauliflower mosaic virus
CRISPR	clustered regularly interspaced short palindromic repeats
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
LOQ	limit of quantification
MS	mass spectrometry
NDF	neutral detergent fibre
NEP	newly expressed protein
NGS	next-generation sequencing
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 sulfate–polyacrylamide gel electrophoresis
SES	standardised effect size
SSI	site-specific integration
TDF	total dietary fibre
TDI	total daily intake
T-DNA	transfer-deoxyribonucleic acid
UTR	untranslated region

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CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

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European Commission

QUESTION NUMBER

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PANEL MEMBERS

Ewen Mullins, Jean-Louis Bresson, Tamas Dalmay, Ian Crawford Dewhurst, Michelle M Epstein, Leslie George Firbank, Philippe Guerche, Jan Hejatko, Hanspeter Naegeli, Francisco Javier Moreno, Fabien Nogué, Nils Rostoks, Jose Juan Sánchez Serrano, Giovanni Savoini, Eve Veromann and Fabio Veronesi.

REFERENCES

Alimentarius, C. (2009). *Foods derived from modern biotechnology*. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme. https://www.fao.org/docrep/011/a1554e/a1554e00.htm

Boeckman, C. J., Ballou, S., Gunderson, T., Huang, E., Linderblood, C., Olson, T., Stolte, B., LeRoy, K., Walker, C., Wang, Y., Woods, R., & Zhang, J. (2022). Characterization of the spectrum of activity of IPD079Ea: A protein derived from *Ophioglossum pendulum* (Ophioglossales: Ophioglossaceae) with activity against Western corn rootworm [*Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae)]. *Journal of Economic Entomology*, *115*(5), 1531–1538. https://doi.org/10.1093/jee/toac079

Breiteneder, H., & Mills, E. N. (2005). Molecular properties of food allergens. *Journal of Allergy and Clinical Immunology*, *115*, 14–23.

Costa, J., Bavaro, S. L., Benede, S., Diaz-Perales, A., Bueno-Diaz, C., Gelencser, E., Klueber, J., Larre, C., Lozano-Ojalvo, D., Lupi, R., Mafra, I., Mazzucchelli, G., Molina, E., Monaci, L., Martın-Pedraza, L., Piras, C., Rodrigues, P. M., Roncada, P., Schrama, D., … Holzhauser, T. (2022). Are physicochemical properties shaping the allergenic potency of plant allergens? *Clinical Reviews in Allergy and Immunology*, *62*, 37–63. https://doi.org/10.1007/s12016-020-08810-9

Eastham, K., & Sweet, J. (2002). *Genetically modified organisms (GMOs): The significance of gene flow through pollen transfer*. European Environment Agency, environmental issue report, 28, 1–75. http://www.eea.europa.eu/publications/environmental_issue_report_2002_28

EFSA (European Food Safety Authority). (2009). Consolidated presentation of the joint scientific opinion of the GMO and BIOHAZ panels on the "use of antibiotic resistance genes as marker genes in genetically modified plants" and the scientific opinion of the GMO panel on "consequences of the opinion on the use of antibiotic resistance genes as marker genes in genetically modified plants on previous EFSA assessments of individual GM plants". *EFSA Journal*, *7*(6), 1108. https://doi.org/10.2903/j.efsa.2009.1108

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

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

EFSA (European Food Safety Authority). (2015). Reasoned opinion on the review of the existing maximum residue levels (MRLs) for glufosinate according to article 12 of regulation (EC) No 396/2005. *EFSA Journal*, *13*(1), 3950. https://doi.org/10.2903/j.efsa.2015.3950

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

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

EFSA (European Food Safety Authority), Paraskevopoulos, K., Ramon, M., Dalmay, T., du Jardin, P., Casacuberta, J., Guerche, P., Jones, H., Nogué, F., Robaglia, C., & Rostoks, N. (2018). *Explanatory note on the determination of newly expressed protein levels in the context of genetically modified plant applications for EU market authorisation*. EFSA supporting publication 2018; 15(8):EN-1466, 13 pp. https://doi.org/10.2903/sp.efsa.2018.EN-1466

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

EFSA (European Food Safety Authority), Devos, Y., Guajardo, I. M., Alvarez, F., & Glanville, J. (2019b). *Explanatory note on literature searching conducted in the context of GMO applications for (renewed) market authorisation and annual post-market environmental monitoring reports on GMOs authorised in the EU market*. EFSA supporting publications 2019:EN-1614, 62 pp. https://doi.org/10.2903/sp.efsa.2019.en-1614

EFSA (European Food Safety Authority), Devos, Y., Aiassa, E., Muñoz-Guajardo, I., Messéan, A., & Mullins, E. (2022). Statement on the update of environmental risk assessment conclusions and risk management recommendations of EFSA (2016) on EU teosinte. *EFSA Journal*, *20*(3), 7228. https://doi.org/10.2903/j.efsa.2022.7228

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

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

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2010c). Scientific opinion on the assessment of allergenicity of GM plants and micro-organisms and derived food and feed. *EFSA Journal*, *8*(7), 1700. https://doi.org/10.2903/j.efsa.2010.1700

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

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

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2012). Scientific opinion on application (EFSA-GMO-DE-2010-82) for the placing on the market of insect resistant genetically modified maize MIR162 for food and feed uses, import and processing under regulation (EC) No 1829/2003 from Syngenta. *EFSA Journal*, *10*(6), 27. https://doi.org/10.2903/j.efsa.2012.2756

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

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2015b). Scientific opinion on application (EFSA-GMO-DE-2011-95) for the placing on the market of genetically modified maize 5307 for food and feed uses, import and processing under regulation (EC) No 1829/2003 from Syngenta crop protection AG. *EFSA Journal*, *13*(5), 4083. https://doi.org/10.2903/j.efsa.2015.4083

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli, H., Birch, A. N., Casacuberta, J., De Schrijver, A., Gralak, M. A., Guerche, P., Jones, H., Manachini, B., Messean, A., Nielsen, E. E., Nogue, F., Robaglia, C., Rostoks, N., Sweet, J., Tebbe, C., Visioli, F., Wal, J.-M., Eigenmann, P., … Fernandez Dumont, A. (2017). Guidance on allergenicity assessment of genetically modified plants. *EFSA Journal*, *15*(5), 4862. https://doi.org/10.2903/j.efsa.2017.4862

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Casacuberta, J., Nogué, F., Naegeli, H., Birch, A. N., De Schrijver, A., Gralak, M. A., Guerche, P., Manachini, B., Messéan, A., Nielsen, E. E., Robaglia, C., Rostoks, N., Sweet, J., Tebbe, C., Visioli, F., Wal, J.-M., Moxon, S., Schneeberger, K., … Jones, H. (2018). Scientific opinion on the technical note on the quality of DNA sequencing for the molecular characterisation of genetically modified plants. *EFSA Journal*, *16*(7), 5345. https://doi.org/10.2903/j.efsa.2018.5345

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli, H., Bresson, J.-L., Dalmay, T., Dewhurst, I. C., Epstein, M. M., Firbank, L. G., Guerche, P., Hejatko, J., Moreno, F. J., Mullins, E., Nogué, F., Rostoks, N., Sánchez Serrano, J. J., Savoini, G., Veromann, E., Veronesi, F., Álvarez, F., Ardizzone, M., … Raffaello, T. (2020). Scientific opinion on the assessment of genetically modified maize MZIR098 for food and feed uses, under regulation (EC) No 1829/2003 (application EFSA-GMO-DE-2017-142). *EFSA Journal*, *18*(6), 6171. https://doi.org/10.2903/j.efsa.2020.6171

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli, H., Bresson, J.-L., Dalmay, T., Dewhurst, I. C., Epstein, M. M., Firbank, L. G., Guerche, P., Hejatko, J., Moreno, F. J., Mullins, E., Nogue, F., Rostoks, N., Sànchez Serrano, J. J., Savoini, G., Veromann, E., Veronesi, F., & Fernandez Dumont, A. (2021). Statement on *in vitro* protein digestibility tests in allergenicity and protein safety assessment of genetically modified plants. *EFSA Journal*, *19*(1), 6350. https://doi.org/10.2903/j.efsa.2021.6350

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Mullins, E., Bresson, J.-L., Dalmay, T., Dewhurst, I. C., Epstein, M. M., Firbank, L. G., Guerche, P., Hejatko, J., Moreno, F. J., Naegeli, H., Nogué, F., Rostoks, N., Sánchez Serrano, J. J., Savoini, G., Veromann, E., Veronesi, F., Ardizzone, M., Camargo, A. M., … Streissl, F. (2022a). Scientific opinion on the assessment of genetically modified maize MON 87429 for food and feed uses, under regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2019-161). *EFSA Journal*, *20*(11), 7589. https://doi.org/10.2903/j.efsa.2022.7589

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Mullins, E., Bresson, J.-L., Dalmay, T., Dewhurst, I. C., Epstein, M. M., Firbank, L. G., Guerche, P., Hejatko, J., Moreno, F. J., Naegeli, H., Nogué, F., Rostoks, N., Sánchez Serrano, J. J., Savoini, G., Veromann, E., Veronesi, F., Camargo, A. M., Goumperis, T., … Streissl, F. (2022b). Scientific opinion on the assessment of genetically modified maize MIR162 for renewal authorisation under regulation (EC) No 1829/2003 (application EFSA-GMO-RX-025). *EFSA Journal*, *20*(9), 7562. https://doi.org/10.2903/j.efsa.2022.7562

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

Foo, A. C. Y., & Mueller, G. A. (2021). Abundance and stability as common properties of allergens. *Frontiers in Allergy*, *2*, 769728.

Gruber, S., Colbach, N., Barbottin, A., & Pekrun, C. (2008). Post-harvest gene escape and approaches for minimizing it. *CAB Reviews*, *3*, 1–7.

Helm, R. M. (2001). *Topic 5: Stability of known allergens (digestive and heat stability). Report of a joint FAO, WHO expert consultation on allergenicity of food derived from biotechnology, 22–25, January 2001*. Food and Agriculture organisation of the United Nations (FAO).

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

Le Corre, V., Siol, M., Vigouroux, Y., Tenaillon, M. I., & Dély, C. (2020). Adaptive introgression from maize has facilitated the establishment of teosinte as a noxious weed in Europe. *Proceedings of the National Academy of Sciences of the United States of America*, *117*, 25618–25627.

Lecoq, E., Holt, K., Janssens, J., Legris, G., Pleysier, A., Tinland, B., & Wandelt, C. (2007). General surveillance: Roles and responsibilities the industry view. *Journal für Verbraucherschutz und Lebensmittelsicherheit-Journal of Consumer Protection and Food Safety*, *2*(S1), 25–28.

Li, Y., Tran, A. H., Danishefsky, S. J., & Tan, Z. (2019). Chemical biology of glycoproteins: From chemical synthesis to biological impact. *Methods in Enzymology*, *621*, 213–229.

Meier, U. (2001). *Growth stages of mono-and dicotyledonous plants* (2nd ed.). BBCH Monograph. Federal Biological Research Centre for Agriculture and Forestry.

OECD (Organisation for Economic Co-operation and Development). (2002). *Consensus document on compositional considerations for new varieties of maize (Zea mays): Key food and feed nutrients, anti-nutrients and secondary plant metabolites*. Series on the Safety of Novel Food and Feeds (ENV/JM/MONO(2002)25), 6, 1–42.

OECD (Organisation for Economic Co-operation and Development). (2003). *Consensus document on the biology of Zea mays subsp. mays (maize)*. Series on Harmonisation of Regulatory Oversight in Biotechnology (ENV/JM/MONO(2003)11), 27, 1–49.

OECD (Organisation for Economic Co-operation and Development). (2008). *Test no. 407: Repeated dose 28-day oral toxicity study in rodents, OECD guidelines for the testing of chemicals, section 4*. OECD Publishing. https://doi.org/10.1787/9789264070684-en

OECD (Organisation for Economic Co-operation and Development). (2013). *Guidance document on residues in livestock*. In: Series on Pesticides No 73ENV/JM/MONO(2013), 8, 4 September 2013.

OECD (Organisation for Economic Co-operation and Development). (2018). *OECD guideline for the testing of chemicals – Test no. 408: Repeated dose 90-day oral toxicity study in rodents*. OECD Publishing.

Palaudelmàs, M., Peñas, G., Melé, E., Serra, J., Salvia, J., Pla, M., Nadal, A., & Messeguer, J. (2009). Effect of volunteers on maize gene flow. *Transgenic Research*, *18*, 583–594.

Pascher, K. (2016). Spread of volunteer and feral maize plants in Central Europe: Recent data from Austria. *Environmental Sciences Europe*, *28*, 30.

Polevoda, B., & Sherman, F. (2000). Na-terminal acetylation of eukaryotic proteins. *Journal of Biological Chemistry*, *275*, 36479–36482.

Sys, C., Van Ranst, E., Debaveye, J., & Beernaert, F. (1993). *Land evaluation. Part III: Crop requirements*. Agricultural publication no. 7. Brussels, General Administration for Development Cooperation. 199 pp.

Trtikova, M., Lohn, A., Binimelis, R., Chapela, I., Oehen, B., Zemp, N., Widmer, A., & Hilbeck, A. (2017). Teosinte in Europe – Searching for the origin of a novel weed. *Scientific Reports*, *71*, 1560.

Windels, P., Alcalde, E., Lecoq, E., Legris, G., Pleysier, A., Tinland, B., & Wandelt, C. (2008). General surveillance for import and processing: The EuropaBio approach. *Journal of Consumer Protection and Food Safety*, *3*(Suppl. 2), 14–16.

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 DP915635 for humans, animal or the environment.

Study identification	Title
PHI-2020-005	(2021) An 8-week channel catfish (*Ictalurus punctatus*) dietary tolerance study of maize grain containing event DP-915635-4
PHI-2020-006	(2020) Nutritional Equivalency Study of Maize Grain DP-915635-4 – Poultry Feeding Study
PHI-2020-010	(2020) Evaluation of germination and Viability of a Maize Line Containing the Event DP-915635-4
PHI-R115-Y20	(2020) Field-based corn rootworm efficacy of maize containing event DP-915635-4 from the 2020 growing season

APPENDIX B

List of relevant publications identified by the applicant through literature searches (January 2010–June 2023)

Reference
Anderson JA, Mickelson J, Fast BJ, Smith N, Pauli RC, Walker C, 2023. Genetically modified DP915635 maize is agronomically and compositionally comparable to non-genetically modified maize. *GM Crops & Food* 14(1), 1–8
Carlson AB, Mathesius CA, Ballou S, Fallers MN, Gunderson TA, Hession A, Mirsky H, Stolte B, Zhang J, Woods RM, Herman RA, Roper JM, 2022. Safety assessment of the insecticidal protein IPD079Ea from the fern, Ophioglossum pendulum. *Food and Chemical Toxicology* 166, 9
Christ B, Hochstrasser R, Guyer L, Francisco R, Aubry S, Hortensteiner S and Weng J-K, 2017. Non-specific activities of the major herbicide-resistance gene BAR. *Nature Plants* 3, 937–945
Herman RA, Hou ZL, Mirsky H, Nelson ME, Mathesius CA and Roper JM, 2021. History of safe exposure and bioinformatic assessment of phosphomannose-isomerase (PMI) for allergenic risk. *Transgenic Research* 30, 201–206
Schafer BW, Embrey SK and Herman RA, 2016. Rapid simulated gastric fluid digestion of in- seed/grain proteins expressed in genetically engineered crops. *Regulatory Toxicology and Pharmacology* 81, 106–112

APPENDIX C

Statistical analysis and statistically significant findings in the 28-day toxicity study in mice on the microbially produced IPD079Ea protein and 90-day toxicity study in rats on maize DP915635

C.1 | Statistical analysis of the 28-day study on the microbially produced IPD079Ea protein in mice

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 each of the three test diet groups (high, medium and low IPD079Ea protein groups) separately with the Basal Diet Control group.

The analysis was performed for male and female mice separately at 5% level of significance. Continuous data were analysed using a linear mixed model (LMM): pairwise comparisons, between each test and control group (separately for each sex) were evaluated with the *p*-values for the fixed effect (diet group). Non-continuous endpoints such as some of the FOB parameters were analysed using either Fisher's exact tests, Freeman–Halton tests or Wilcoxon rank-sum tests.

Ranges from historical control data were provided to aid the assessment of statistically significant differences between the test and control diet groups. Missing data were considered by the Panel and found not to have an impact on the results.

TABLE C.1 Statistically significant findings in 28-day study on microbially produced IPD079Ea protein in mice.

Statistically significant parameter/endpoint	Finding	GMO Panel interpretation
Mean body weight gains	Variations (increased and decreased) over some periods	Terminal body weights similar across all groups (within 4%). Within normal variation. Not an adverse effect of treatment
Food consumption	Increased over some periods (up to 20%)	Increased food consumption is not adverse in isolation. No dose relationship. No impact on final body weight. Not an adverse effect of treatment
FOB and motor activity		
Motor activity	Higher least squares mean overall total activity counts in the IPD079Ea low group males compared to the BSA control group at the Week 4 evaluation. Higher least squares mean overall total and ambulatory activity counts in the IPD079Ea low group females compared to the basal diet control group at the Week 4 evaluation	Within normal variation. No significant differences versus the respective basal or BSA diets groups. Pre-test values for the low dose groups were higher than control pre-test values. No dose response. Not an adverse effect of treatment
Haematology		
Mean lymphocyte count	Lower (30%) in the IPD079Ea high group females when compared to the basal diet control group	Small magnitude. No significant differences versus the BSA group. Within normal variation. No effects on other related haematological or histological endpoints. Not an adverse effect of treatment
Coagulation		
Mean prothrombin time	Lower (10%) in the IPD079Ea mid group males when compared to the BSA control group	No differences versus the basal diet group. Not significant at the top dose. Within normal variation (test group values within control ranges). Not an adverse effect of treatment
Clinical chemistry		
Mean glucose level	Higher (18%) in the IPD079Ea mid group males when compared to the basal diet control group	No significant differences versus the BSA group Small magnitude. Within normal variation. Not an adverse effect of treatment
Mean cholesterol level	Higher (30%–40%) in the IPD079Ea high group males (statistically significant) and mid-group males (not significant) when compared to the basal diet control and BSA control groups	The increases in cholesterol in males at the mid- and high doses cannot be entirely discounted as being related to administration of IPD079Ea. Values in 4 top dose males and 3 mid-dose males were above the concurrent and BSA control ranges but the magnitude was small (< 25%; max 219 mg/dL versus 176 mg/dL). There were no adverse histopathological findings in animals receiving IPD079Ea, including in organs associated with cholesterol such as the liver. Overall, it is concluded that the increases in cholesterol levels in mice administered IPD079Ea are not adverse based on the magnitude of the change and the absence of other findings

TABLE C.1 (Continued)

Statistically significant parameter/endpoint	Finding	GMO Panel interpretation
Organ weight		
Pituitary weight, absolute and relative to brain weight	Higher (40%) in the IPD079Ea low group males compared to the basal diet control group	No dose response. Not significant against BSA control. No associated histopathology findings. Not an adverse effect of treatment
Brain weight, absolute	Increased (5%) in high-dose females versus BSA	Small magnitude. Not significant against basal control No associated histopathological findings. Within normal variation (top dose values within basal and BSA control ranges). Not an adverse effect of treatment

C.2 | Statistical analysis of the 90-day study on maize DP915635 in rats

The following endpoints were statistically analysed: body weights, cumulative body weight changes, food consumption, clinical pathology values (as applicable), absolute and relative organ weights, FOB data, locomotor activity, and histopathological data. For all continuous endpoints, the applicant reported mean, standard deviation in terms of the SES of each dose group for each sex, variable, and period or time interval.

The main statistical analysis (at the 5% level of significance) compared rats consuming the test diets (at low and high dose) with those consuming the control diet. Continuous data were investigated separately for each variable with a LMM; pairwise comparisons, between each test and control group (separately for each sex, if necessary) were performed using linear contrasts between diet effects, both across genders and within each gender (treatment, sex and their interaction were defined as fixed effects). For the endpoints with the cage as experimental unit (such as food consumption) the model included block (per sex) as a random effects; for the other endpoints (with the individual rat as experimental unit) an additional random effect was included to take into account the cage effect. For endpoints measured on an ordinal scale (with the exception of PH), the comparisons were performed using Fisher's exact test. For all the models, in case the sex-by-treatment interaction was significant (and in any case for sex-specific parameters) a sex-specific analysis was performed. Historical control data were provided but were not used for the assessment of statistically significant outcomes.

TABLE C.2 Statistically significant findings in 90-day study on maize DP915635 in rats.

Statistically significant parameter/endpoint	Finding	GMO Panel interpretation
Body weight and body weight gain	Increases and decreases over different periods of the study. Decreased (6%) in the low-dose groups combined at Day 91	Small magnitude. No dose response. Terminal body weight in high-dose groups combined was within 1% of controls. Not an adverse effect of treatment
Food consumption and food efficiency	Variations over different periods of the study. Food consumption reduced overall in top-dose females (10%) Food efficiency reduced overall in low-dose groups combined (10%)	Small magnitude. Within normal variation. No impact on final body weight. Not an adverse effect of treatment
Motor activity	Variations in duration of movement and number of movements at individual time points. Number of movements decreased overall in low-dose males (34%)	No impact on total duration of movement. No dose response for number of movements. Within normal variation. Not an adverse effect of treatment
Haematology Mean haemoglobin concentration (HGB)	Reduced (2%) in the low-dose groups combined and low-dose females	Small magnitude. No dose response. Not an adverse effect of treatment
Haematology Mean corpuscular haemoglobin concentration (MCHC)	Reduced (1.5%) in low-dose females	Small magnitude. No dose response. Not an adverse effect of treatment
Haematology – Mean absolute reticulocyte count (ARET)	Increased (20%) in both the female groups and in both the groups combined (10%)	Within normal variation as shown by reference diet animals. No changes in RBC count. Not an adverse effect of treatment
Haematology – Mean absolute eosinophil concentration (AEOS)	Reduced in low-dose females (31%) and both low-dose groups combined (16%)	Not seen at top dose (5% higher than controls). Not an adverse effect of treatment

(Continues)

TABLE C.2 (Continued)

Statistically significant parameter/endpoint	Finding	GMO Panel interpretation
Coagulation	Mean prothrombin time (PT) was longer in females in the low- (6%) and high-dose (5%) groups	Small magnitude. No dose response and no effect in males. Within physiological range and normal variation. Not an adverse effect of treatment
Clinical chemistry – Alkaline phosphatase (ALKP)	Increased in the low-dose groups combined (13%) and low-dose females (27%)	No dose response. Within normal variation as shown by reference diet values. No related clinical chemistry or histopathology changes. Not an adverse effect of treatment
Clinical chemistry – Cholesterol and lipoproteins	Mean cholesterol concentration (CHOL), high-density lipoprotein concentration (HDL) and non-high-density lipoprotein concentration (nHDL) were all lower in the low-dose groups combined (16%–22%) and in low-dose females (21%–32%)	No dose response. Not adverse in isolation. Within normal variation as shown by reference diet values. Not an adverse effect of treatment
Clinical chemistry – Albumin and total protein	Increased in the high-dose groups combined (3%–4%) and high-dose females (5%–7%)	Low magnitude. Within normal variation as shown by reference diet values. Not an adverse effect of treatment
Clinical chemistry – Calcium	Decreased (3%) in both the high dose groups combined and in high-dose females (4%)	Low magnitude. Within normal variation as shown by reference diet values. Not an adverse effect of treatment
Clinical chemistry – Total bile acids	Decreased (27%) in low-dose females	Reduction is not adverse in isolation. No dose response. Not seen in males, where there was a non-significant increase. Within normal variation. Not an adverse effect of treatment
Hormones evaluations T4	Increased in both the top-dose groups combined (12%) and in top-dose males (12%)	Low magnitude. Within normal variation as shown by reference diet values. No changes in thyroid histopathology. Not an adverse effect of treatment
Urinalysis – pH	Mean urine pH was higher in the low-dose female group	Mean value was pH 7.0 and range (6.5–7.5) are within the concurrent control range. No dose response. Not an adverse effect of treatment
Urinalysis – URO	Mean urobilinogen (URO) was lower in the low- and high-dose male groups	Control group looks to be the outlier with several high values of 1.0. Both test groups are all 0.2. All test group values are within the control group range. Not an adverse effect of treatment
Organ weight – Brain weight relative to body weight	Increased (6%) in both the low-dose groups combined	Low magnitude. No dose response. Within normal variation. No associated pathology. Not adverse
Organ weight – Kidney weight	Decreased (6%) in both the low-dose groups combined and in low-dose males (8%)	Low magnitude. No dose response. Within normal variation. No associated pathology, urinalysis or clinical chemistry findings
Organ weight – Thymus weight relative to body weight	Increased (16%) in high-dose females	No significant change in absolute weight or relative to brain weight. In males, there was a non-significant decrease of 16%. Within normal variation as shown by the concurrent control range and reference diet values. No associated haematology or pathology findings. Not an adverse effect of treatment
Organ weight – ovaries relative to body weight	Increased (15%) in low-dose females	No dose response. Within normal variation, all values with concurrent control range. No associated histopathology findings. Not an adverse effect of treatment
Organ weight – pituitary absolute, relative to body weight and brain weight	One or more results reduced in both the high-dose groups combined (10%–13%) in high-dose females (14%–19%), and all the low-dose groups (8%–12%)	Low magnitude. Within normal variation. No dose response in males. No associated histopathology findings. Not an adverse effect of treatment

APPENDIX D

Animal dietary exposure

TABLE D.1 Dietary exposure to IPD079Ea protein (mg/kg bw per day) in livestock, based on the consumption of maize grain and forage.

			IR (%)				
	BW (kg)	TDI feed (kg DM/animal)	Grain (G)	Forage (F)	G	F	G+F
Broiler	1.7	0.12	70	NA	0.014	–	–
Layer	1.9	0.13	70	10	0.014	0.0021	0.016
Turkey	7	0.50	50	NA	0.010	–	–
Breeding pigs	260	6	70	20	0.0047	0.0014	0.0061
Finishing pigs	100	3	70	NA	0.0061	–	–
Beef cattle[a]	500	12	80	80	0.0056	0.0060	0.012
Dairy cattle	650	25	30	60	0.0033	0.0072	0.011
Ram/ewe	75	2.5	30	NA	0.0029	–	–
Lamb	40	1.7	30	30	0.0037	0.0040	0.0077

Note: NA indicates that a forage inclusion rate was not provided in the reference and therefore no exposure calculations were done.
[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.

			IR (%)				
	BW (kg)	TDI feed (kg DM/animal)	Grain (G)	Forage (F)	G	F	G+F
Broiler	1.7	0.12	70	NA	0.54	–	–
Layer	1.9	0.13	70	10	0.53	0.10	0.63
Turkey	7	0.50	50	NA	0.39	–	–
Breeding pigs	260	6	70	20	0.18	0.069	0.25
Finishing pigs	100	3	70	NA	0.23	–	–
Beef cattle[a]	500	12	80	80	0.21	0.29	0.50
Dairy cattle	650	25	30	60	0.13	0.35	0.47
Ram/ewe	75	2.5	30	NA	0.11	–	–
Lamb	40	1.7	30	30	0.14	0.19	0.33

Note: NA indicates that a forage inclusion rate was not provided in the reference and therefore no exposure calculations were done.
[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.

			IR (%)				
	BW (kg)	TDI feed (kg DM/animal)	Grain (G)	Forage (F)	G	F	G+F
Broiler	1.7	0.12	70	NA	0.38	–	–
Layer	1.9	0.13	70	10	0.37	0.12	0.49
Turkey	7	0.50	50	NA	0.28	–	–
Breeding pigs	260	6	70	20	0.12	0.078	0.20
Finishing pigs	100	3	70	NA	0.16	–	–
Beef cattle[a]	500	12	80	80	0.15	0.33	0.47
Dairy cattle	650	25	30	60	0.089	0.39	0.48
Ram/ewe	75	2.5	30	NA	0.077	–	–
Lamb	40	1.7	30	30	0.098	0.22	0.31

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