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SCIENTIFIC OPINION

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Assessment of genetically modified maize DP23211 for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2019-163)

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Abstract

Genetically modified maize DP23211 was developed to confer control of certain coleopteran pests and tolerance to glufosinate-containing herbicide. These properties were achieved by introducing the *pmi*, *mo-pat*, *ipd072Aa* and *DvSSJ1* 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 DP23211 and its conventional counterpart needs further assessment, except for those in levels of histidine, phenylalanine, magnesium, phosphorus and folic acid in grain, which do not raise safety and nutritional concerns. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the IPD072Aa, PAT and PMI proteins and the DvSSJ1 dsRNA and derived siRNAs newly expressed in maize DP23211, and finds no evidence that the genetic modification impacts the overall safety of maize DP23211. In the context of this application, the consumption of food and feed from maize DP23211 does not represent a nutritional concern in humans and animals. Therefore, no post-market monitoring of food/feed is considered necessary. In the case of accidental release of viable maize DP23211 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 DP23211. The GMO Panel concludes that maize DP23211 is as safe as its conventional counterpart and the tested non-GM reference varieties with respect to potential effects on human and animal health and the environment.

KEYWORDS

DP23211, DvSSJ1, genetic engineering, GM, import and processing, IPD072Aa, maize (*Zea mays*), PAT, PMI

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SUMMARY

Following the submission of application EFSA-GMO-NL-2019-163 under Regulation (EC) No 1829/2003 from Corteva Agriscience Belgium B.V. (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) maize (*Zea mays* L.) DP23211 according to Regulation (EU) No 503/2013. Maize DP23211 was developed to confer control of certain coleopteran pests and tolerance to glufosinate-containing herbicide. The scope of application EFSA-GMO-NL-2019-163 is for import, processing, and food and feed uses within the European Union (EU) of maize DP23211 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 DP23211 according to the scope of the application EFSA-GMO-2019-163. The GMO Panel conducted the assessment of maize DP23211 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 DP23211 contains a single insert consisting of one copy of the *pmi*, *mo-pat*, *ipd072Aa* and *DvSSJ1* dsRNA expression cassettes. The quality of the sequencing methodology and datasets was assessed by the EFSA GMO Panel and is in compliance to the requirements listed in the EFSA Technical Note. 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 *in planta* RNAi off-target search, performed with the sequence of the DvSSJ1 dsRNA, does not provide indication for an off-target effect that would need further safety assessment. The stability of the inserted DNA and of the introduced trait is confirmed over several generations. The methodology used to quantify the levels of the IPD072Aa, PAT and PMI proteins is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of plant- and microbe-derived IPD072Aa proteins, indicate that these proteins are equivalent, and the microbe-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 DP23211 and its conventional counterpart needs further assessment, except for those in levels of histidine, phenylalanine, magnesium, phosphorus and folic acid in grain, which do not raise safety and nutritional concerns. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the IPD072Aa, PAT and PMI proteins and the DvSSJ1 dsRNA and derived siRNAs as expressed in maize DP23211. The GMO Panel finds no evidence that the genetic modification impacts the overall safety of maize DP23211. In the context of this application, the consumption of food and feed from maize DP23211 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that maize DP23211 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 DP23211 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 DP23211.

The GMO Panel considered the overall quality of the performed literature searches acceptable. Based on the relevant publications identified through the literature searches, the GMO Panel does not identify any safety issue pertaining to the intended uses of maize DP23211.

The GMO Panel concludes that maize DP23211 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-2019-163 is for food and feed uses, import and processing of maize DP23211 and does not include cultivation in the European Union (EU). Maize DP23211 was developed to confer control of certain coleopteran pests and tolerance to glufosinate-containing herbicide.

1.1 | Background

On 13 December 2019, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands application EFSA-GMO-NL-2019-163 for authorisation of maize DP23211 (Unique Identifier DP-Ø23211-2), submitted by Corteva Agriscience Belgium B.V. (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003.[1] Following receipt of application EFSA-GMO-NL-2019-163, EFSA informed EU Member States and the European Commission, and made the application available to them. Simultaneously, EFSA published the summary of the application.[2]

EFSA checked the application for compliance with the relevant requirements of 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 16 April 2020, EFSA declared the application valid.

From the validity date, EFSA and its 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-2019-163. Such time limit was extended whenever EFSA and/or its GMO Panel requested supplementary information to the applicant. According to Regulation (EC) No 1829/2003, any supplementary information provided by the applicant during the risk assessment was made available to the EU Member States and to 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-2019-163 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 DP23211 in the context of its scope as defined in application EFSA-GMO-NL-2019-163.

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

2 | DATA AND METHODOLOGIES

2.1 | Data

The GMO Panel based its scientific risk assessment of maize DP23211 on the valid application EFSA-GMO-NL-2019-163, additional information provided by the applicant during the risk assessment, relevant scientific comments submitted by the Member States and relevant peer-reviewed scientific publications. As part of this comprehensive information package, the GMO Panel received additional unpublished studies submitted by the applicant in order to comply with the specific provisions of Regulation (EU) No 503/2013. A list of these additional unpublished studies is provided in Appendix A.

2.2 | Methodologies

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

[1]Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.

[2]Available online: https://open.efsa.europa.eu/questions/EFSA-Q-2020-00786

[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, p. 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, p. 1–38.

[5]These particulars are available online at: https://open.efsa.europa.eu/study-inventory/EFSA-Q-2019-00807.

For this application, in the context of the contracts OC/EFSA/GMO/2018/04, OC/EFSA/GMO/2018/02, OC/EFSA/GMO/2020/01 and EOI/EFSA/SCIENCE/2020/01–CT02 GMO, 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 DP23211.

3 | ASSESSMENT

3.1 | Introduction

Maize DP23211 express DvSSJ1 double-stranded ribonucleic acid (dsRNA) and the IPD072Aa protein, both for control of certain coleopteran pests, as well as the phosphinothricin acetyltransferase (PAT) protein to confer tolerance to glufosinate-containing herbicide, and the phosphomannose isomerase (PMI) protein that was used as a selectable marker.

3.2 | Systematic literature review[6]

The GMO Panel assessed the applicant's literature searches on maize DP23211, which include a scoping review, according to the guidelines given in EFSA (2010, 2019b).

A systematic review as referred to in Regulation (EU) No 503/2013 has not been provided in support to the risk assessment of application EFSA-GMO-NL-2019-163. Based on the outcome of the scoping review, the GMO Panel agrees that there is limited value of undertaking a systematic review for maize DP23211 at present.

The GMO Panel considered the overall quality of the performed literature searches acceptable. The literature searches identified nine relevant publications on maize DP23211 (Appendix B). Based on the relevant publications,[7] the GMO Panel does not identify any safety issues pertaining to the intended uses of maize DP23211.

3.3 | Molecular characterisation[8]

3.3.1 | Transformation process and vector constructs

Maize DP23211 was developed by site-specific integration (SSI) in two sequential steps.

1. Microprojectile co-bombardment and a I-Cre endonuclease-mediated targeted insertion process to insert a 'landing pad', at a specific location of the maize genome, using three plasmids (PHP56614, PHP21139 and PHP31729).
2. In the second step, a selected maize transformant with the integrated 'landing pad' was co-cultured with a disarmed *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*)-mediated transformation to insert the intended expression cassettes into the landing pad in the maize genome. Explants were co-cultured with a disarmed *A. tumefaciens* strain ABI containing the vector PHP74643.

After the first step, the I-Cre endonuclease produces a double-strand break in a targeted location in the maize genome. The break induces a homology-directed repair mechanism, allowing a recombination between the zm-SEQ9 and zm-SEQ8 sequences from PHP56614 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 maize (*Z. mays)* ubiquitin (*ubiZM1*) 5'-UTR intron and promoter and the *nptII* gene flanked by the flippase recombination sites FRT1 and FRT87. Two more plasmids, PHP21139 and PHP31729 expressed the WUS protein and the ODP2 protein, respectively, to improve regeneration. The *I-CreI*, the *wus* and the *odp2* genes were all transiently expressed, without integration in the plant genome.

The plasmid PHP56614, used to insert the landing pad, contains two expression cassettes between the right and left border of the T-DNA, containing the following genetic elements:

– The I-CreI cassette consists of the promoter region from the maize ubiquitin gene 1 of *Z. mays* (*ubiZM1*) including the 5' untranslated region (5' UTR) and intron, the maize-optimised coding sequence of the I-Cre endonuclease gene from *Chlamydomonas reinhardtii*, and the terminator region from the *pin*II gene of *Solanum tuberosum*. The cassette is flanked by *loxP* sites.

[6]Dossier: Part II – Section 7; additional information: 22/8/2023 and 8/11/2023.

[7]The GMO Panel noted that in the updated literature search, four publications were retrieved but not extracted as relevant by the applicant: Hu et al. (2020), describing the mode of action of DvSSJ1; Jiménez-Juárez et al. (2023), describing the mode of action of the IPD072Aa protein; Boeckman et al. (2020), describing the spectrum of insecticidal activity of the IPD072Aa protein and Boeckman et al. (2021), performing an ERA on NTOs of the IPD072Aa protein and DvSSJ1. The GMO Panel considered the articles relevant but concluded that they do not add new information that would raise concerns for safety. The mode of action of IPD072Aa endpoint is discussed in the Scientific Opinion using information from the applicant's main dossier and additional information on 5/7/2021 (see Section 3.5.3.1).

[8]Dossier: Part II – Section 1.2; additional information: 9/10/2020, 11/3/2021, 7/7/2021, 10/11/2021 and 15/9/2023.

– 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*, and the terminator region from the *pinII* gene of *Solanum tuberosum*. The *nptII* coding region and the terminator are flanked by the FRT1 and FRT87 recombination sites from *Saccharomyces cerevisiae*, intended to facilitate recombination after the second transformation step with plasmid PHP74643. The entire cassette is flanked by the zm-SEQ9 and zm-SEQ8 sequences, homologous to the maize genome and used to drive the insertion of the landing pad by homologous recombination.

The plasmid PHP21139 contains one expression cassette, consisting of the following genetic elements:

– The zm-wus2 cassette consists of the promoter region from 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*.

The plasmid PHP31729 contains one expression cassette, consisting of the following genetic elements:

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

The 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 PHP74643. The T-DNA of PHP74643 contains eight expression cassettes in total, four of which flanked by the FRT1 and FRT87 sites. Only the region contained between the FRT1 and FRT87 sites is integrated through a flippase recombination event, replacing the *nptII* cassette introduced with the landing pad in the first step.

The four expression cassettes outside of the FRT1 and FRT87 sites (*zm-wus2*, *zm-odp2*, *mo-Flp* and *DsRed2*), were transiently expressed, without integration into the maize DP23211 genome and consist of the following genetic elements:

– The zm-wus2 cassette consists of the promoter region of the nopaline synthase gene of *A. tumefaciens*, the wus2 coding sequence of the Wuschel2 (*wus2*) gene of *Z. mays* and the terminator region from the *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*.
– The DsRed2 gene cassette consists of the 35S enhancer region from the cauliflower mosaic virus genome (CaMV 35S enhancer), the promoter region of the lipid transfer protein gene (*Ltp2*) from *Hordeum vulgare*, a modified coding sequence of the red fluorescent gene (*DsRed2*) from *Discosoma* sp., and the 35S terminator region from the cauliflower mosaic virus genome (CaMV 35S terminator). An additional copy of the CaMV 35S terminator present between the fourth and fifth cassettes is intended to prevent transcriptional interference between cassettes.

The four expression cassettes between FRT1 and FRT87 sites (*pmi*, *mo-pat*, and *ipd072Aa* and DvSSJ1) that integrated into the maize genome consist of the following genetic elements:

– The pmi expression cassette in PHP74643 lacks the promoter. However, following the integration in the 'landing pad', the *pmi* expression is driven by maize (*Z. mays*) ubiquitin 5′-UTR, intron and *ubiZM1* promoter which are provided by the 'landing pad'. The *pmi* coding sequence is for the phosphomannose isomerase (*pmi*) gene coding sequence from *E. coli*, and the terminator from potato (*Solanum tuberosum*) proteinase inhibitor II gene (*pinII*). An additional terminator from the maize 19-kDa zein gene (*Z19*) is present to prevent transcriptional interference between cassettes.
– The mo-pat expression cassette contains the promoter and intron region of the rice (*Oryza sativa*) actin gene (*os-actin*), the maize codon-optimised coding sequence of the phosphinothricin acetyltransferase gene from *Streptomyces viridochromogenes* (*mo-pat*), and the 35S terminator region from the cauliflower mosaic virus genome (CaMV 35S terminator). Two additional terminators from the sorghum (*Sorghum bicolor*) ubiquitin gene (*sbubi*) and from the γ-kafarin gene (*sb-gkaf*) are present to prevent transcriptional interference between cassettes.
– The ipd072Aa expression cassette contains the promoter region from the banana streak virus of Acuminata Yunnan strain (BSV [AY]), the intron from the maize orthologue of a rice (*Oryza sativa*) hypothetical protein (zm-HPLV9), the coding sequence of an insecticidal protein gene (*ipd072Aa*) from *Pseudomonas chlororaphis* and the terminator region from the *Arabidopsis thaliana* at-T9 gene.
– The DvSSJ1 suppression cassette contains the maize (*Z. mays*) ubiquitin (*ubiZM1*) 5′-UTR intron and promoter, the sequence to express two inverted RNA repeat fragments flanked by stop codons of the smooth septate junction protein 1 gene (*dvssj1*) from western corn rootworm (WCR, *Diabrotica virgifera virgifera*) separated by the intron 1 from the maize

alcohol dehydrogenase gene (*zm-Adh1*) and the terminator region from the maize W64 line 27-kDa gamma zein gene (*Z27G*). Two additional terminators from the *A. thaliana* ubiquitin 14 gene (*UBQ14*) and from the maize *In2-1* gene are present to prevent transcriptional interference between cassettes.

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 DP23211 was performed by next generation sequencing (NGS), junction sequence analysis (JSA), polymerase chain reaction (PCR) and DNA sequence analysis, in order to determine insert copy number, size and organisation of the inserted sequences and to confirm the absence of plasmid backbone sequences.

The approach used is acceptable in terms of coverage and sensitivity. Overall, the quality of the methodology and datasets was assessed by the EFSA GMO Panel and is in compliance to the requirements listed in the EFSA Technical Note (EFSA GMO Panel, 2018a).

NGS and JSA indicated that maize DP23211 contains a single insert, consisting of a single copy of the T-DNA in the same configuration as in the PHP74643 transformation vector. NGS analysis also indicated the absence of vector backbone sequences.

The nucleotide sequence of the entire insert of maize DP23211 together with 1488 bp of the 5′ and 2201 bp of the 3′ flanking regions was determined by Sanger sequencing. The insert of 16176 bp is identical to the T-DNA of PHP74643.

The possible interruption of known endogenous maize genes by the insertion in maize DP23211 was evaluated by bioinformatics analyses of the pre-insertion locus and of the genomic sequences flanking the insert. The results of these analyses do not indicate the interruption of any known endogenous gene in maize DP23211.

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

Bioinformatics analyses of the amino acid sequence of the newly expressed PMI, mo-PAT and IPD072Aa proteins reveal no significant similarities to toxins and allergens. In addition, bioinformatic analyses of the newly created open reading frames (ORFs) within the insert and spanning the junctions between the insert and genomic DNA revealed six ORFs (DP23211_220, DP23211_466, DP23211_724, DP23211_832, DP23211_833 and DP23211_994) showing a sequence similarity to potentially allergenic proteins exceeding a 35% identity over an 80 amino acid window and one short ORF (DP23211_524) that presents an exact 8 amino acid match to known allergens. Two of these ORFs (DP23211_220 and DP23211_466) are within the transcriptional unit of *mo-pat* gene and *ipd072Aa* gene, in the same orientation but in a different reading frame. The remaining ORFs are in reverse orientation, outside transcribed regions, and lack promoters and start codons for proper expression. In conclusion, these analyses indicated that the expression of any ORF showing significant similarities to toxins or allergens is unlikely.

According to Regulation (EU) No 503/2013, when silencing approaches by RNAi have been used in GM plant applications, a bioinformatics analysis to identify potential 'off target' genes is required. The applicants have followed the recommendations by the EFSA GMO Panel for an RNAi off-target search in the plant expressing the dsRNA.[9] None of the maize transcript sequences present in the available databases showed perfect match to any of the siRNAs potentially produced. Few maize transcript sequences had regions matching to those siRNAs with one to four mismatches. Some of these sequences presented matches to more than one potential siRNA (up to seven). The applicant discussed these results, taking into account the potential function of the proteins encoded by the mRNAs matching the siRNAs. The GMO Panel assessed this information and concluded that it does not provide indication for an off-target effect of the DvSSJ1 dsRNA expression that would need further safety assessment.

In order to assess the possibility for horizontal gene transfer (HGT) by homologous recombination (HR), the applicant performed a sequence identity analysis for maize DP23211 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 DP23211 expresses three new proteins: PMI, PAT and IPD072Aa. Given the technical restraints in producing large enough quantities from plants, IPD072Aa was recombinantly produced in *E. coli*. A set of biochemical methods was employed to demonstrate the equivalence between the maize DP23211 and *E. coli*-produced IPD072Aa. 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 DP23211.

[9] https://www.efsa.europa.eu/sites/default/files/event/171025-m.pdf

3.3.3.1 | *PMI protein characterisation*

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and western blot analysis 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 it was not glycosylated. Amino acid sequence analysis of the plant-derived PMI protein by mass spectrometry (MS) and N-terminal sequencing showed that the protein matched the deduced sequence as defined by the *pmi* gene.

3.3.3.2 | *mo-PAT protein characterisation*

SDS–PAGE and western blot analysis 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 it was 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. Poledova & Sherman, 2000) and have been previously assessed by the GMO Panel for newly expressed proteins (EFSA GMO Panel, 2022a).

3.3.3.3 | *IPD072Aa protein characterisation and equivalence*

SDS–PAGE and western blot analysis showed that both plant and *E. coli*-produced IPD072Aa proteins had the expected molecular weight of ~10 kDa and were comparably immunoreactive to IPD072Aa protein-specific antibodies. The molecular weight of the *E. coli*-produced IPD072Aa was also analysed by MS to determine its molecular weight and the results were consistent with the expected molecular mass. Glycosylation detection analysis demonstrated that none of the IPD072Aa proteins were glycosylated. Amino acid sequence of the plant-derived IPD072Aa protein by MS and N-terminal sequencing methods showed that the protein matched the deduced sequence as defined by the *ipd072Aa* gene. In addition, the amino acid sequence analysis data showed that the N-terminal methionine of the plant-produced IPD072Aa was truncated and one His residue from the His-tag used to purify the *E. coli*-produced IPD072Aa remained after cleaving by trypsin treatment. Modifications, such as N-terminal methionine truncation, are common in eukaryotic proteins (Poledova & Sherman, 2000) and have been previously assessed by the GMO Panel for newly expressed proteins (EFSA GMO Panel, 2022a). The applicant was not able to purify a sufficient amount of plant-derived protein for a bioassay; therefore, the activity of insecticidal protein was demonstrated by an assay in which western corn rootworms were fed on whole plants. The functionality of *E. coli*-produced protein was demonstrated by an insect feeding bioassay.

3.3.4 | Information on the expression of the insert

Protein levels of PMI, PAT and IPD072Aa were analysed by enzyme-linked immunosorbent assay (ELISA) in material harvested in a field trial across six locations in the USA and Canada during the 2018 growing season. Samples analysed included root (V6, V9, R1, R4 and R6), leaf (V9, R1, R4 and R6), pollen (R1), forage (R4), whole plant (R1 and R6) and grain (R6) from plants treated and not treated with glufosinate ammonium. The expression levels in pollen ($n = 24$), grains ($n = 24$) and forage ($n = 24$) of the PMI, PAT and IPD072Aa proteins used to estimate human and animal dietary exposure (see Section 3.5.5) are reported in Table 1.

The applicant provided a measure of the levels of DvSSJ1 demonstrating the expression of the dsRNA in different tissues including grain and forage. However, the dsRNA is an intermediate molecule, which is processed by dicer to siRNA molecules, and the levels of dsRNA are not a good proxy for the levels of the active siRNAs in the plant (EFSA GMO Panel, 2018b; Paces et al., 2017). Therefore, the levels of the DvSSJ1 dsRNA were not considered relevant for the risk assessment of maize DP23211-2.

TABLE 1 Mean values, standard deviations and ranges of newly expressed proteins in grain [ng/mg dry weight (dw) and ng/mg fresh weight (fw)], pollen and forage (ng/mg dw) from maize DP23211 ($n = 24$).

	Glufosinate treatment			
	Not treated		Treated	
Tissues	ng/mg dry weight (dw)	ng/mg fresh weight (fw)	ng/mg dry weight (dw)	ng/mg fresh weight (fw)
Grain (R6)				
PMI	5.1[a] ± 1.3[b]	4.1 ± 1.0	4.6 ± 1.3	3.6 ± 1.0
	(2.7–7.4)[c]	(2.2–5.9)	(1.9–7.1)	(1.5–5.7)
PAT	5.4 ± 1.7	4.3 ± 1.4	4.6 ± 1.5	3.6 ± 1.2
	(2.7–8.6)	(2.2–6.9)	(2.1–7.0)	(1.7–5.6)
IPD072Aa	2.6 ± 1.5	2.1 ± 1.2	2.2 ± 1.7	1.8 ± 1.4
	(0.62–5.9)	(0.50–4.7)	(0.26–7.0)	(0.21–5.6)

Tissues	Glufosinate treatment			
	Not treated		Treated	
	ng/mg dry weight (dw)	ng/mg fresh weight (fw)	ng/mg dry weight (dw)	ng/mg fresh weight (fw)
Forage (R4)				
PMI	13 ± 3.2		13 ± 2.5	
	(8.7–24)		(6.8–18)	
PAT	10 ± 2.1		9.9 ± 2.3	
	(6.0–14)		(6.5–15)	
IPD072Aa	22 ± 10		22 ± 11	
	(8.3–39)		(8.9–53)	
Pollen (R1)				
PMI	35 ± 4.7		35 ± 4.9	
	(30–46)		(30–46)	
PAT	60 ± 14		56 ± 13	
	(49–89)		(36–83)	
IPD072Aa	0.76 ± 0.45		0.78 ± 0.46	
	(0.16–1.5)		(0.14–1.5)	

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

3.3.5 | Inheritance and stability of inserted DNA

Genetic stability of the maize DP23211 insert was assessed by Southern analysis of genomic DNA from five generations (T1, T2, T3, T4 and T5). The restriction enzyme/probe combinations used were sufficient to conclude that all the plants tested retained a single copy of the insert and flanking regions.

Inheritance pattern was assessed by quantitative polymerase chain reaction (qPCR)-based segregation analysis and phenotypic analysis (tolerance to glufosinate) from generations T1, T5, BC1F1 in two different genetic backgrounds, BC2F1. The results indicated that the insert was stably inherited in subsequent generations.

Overall, these analyses 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 DP23211 contains a single insert consisting of one copy of the *pmi*, *mo-pat* and *ipd072Aa* and DvSSJ1 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 give rise to safety concerns. The *in planta* RNAi off-target search, performed with the sequence of the DvSSJ1 dsRNA, do not provide indication for an off-target effect that would require further safety assessment. The stability of the inserted DNA and of the introduced trait is confirmed over several generations. The methodology used to quantify the levels of the PMI, PAT and IPD072Aa proteins is considered adequate. The protein characterisation data comparing the structural, biochemical and functional properties of plant- and microbial-derived IPD072Aa proteins indicate that these proteins are equivalent and the microbial-derived proteins can be used in the safety studies.

3.4 | Comparative analysis[10]

3.4.1 | Overview of studies conducted for the comparative analysis

Application EFSA-GMO-NL-2019-163 presents data on agronomic and phenotypic characteristics, as well as on forage and grain composition of maize DP23211 (Table 2). In addition, the application contains a germination and viability study of maize line containing event DP23211 (Appendix A).

[10]Dossier: Part II – Section 1.3; additional information: 08/05/2020, 25/11/2023 and 8/11/2023.

TABLE 2 Main comparative analysis studies to characterise maize DP23211 provided in the application EFSA-GMO-NL-2019-163.

Study focus	Study details	Comparator	Commercial non-GM reference varieties
Agronomic and phenotypic analysis	Field study, 11 sites in the US and 1 site in Canada in 2018[a]	PHEJW × PHR03	14[b]
Compositional analysis	Field study, 7 sites in the US and 1 site in Canada in 2018[a]		

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

[b]Non-GM maize varieties used in the agronomic, phenotypic and compositional field trials, with their corresponding relative maturity indicated in brackets were 2R602 (106); 35A52 (107); BK5883 (108); BK6076 (110); P0604 (106); P0760 (107); P0928 (109); P0993 (109); P1105 (111); P1151 (111); P1197 (111); XL5828 (110); XL5939 (109) and XL6158 (111).

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 DP23211 exposed to the intended herbicide (treated), maize DP23211, the comparator PHEJW × PHR03 and four commercial non-GM maize reference varieties not exposed to the intended herbicide (not treated).

The agronomic, phenotypic and compositional data were analysed as specified by the EFSA GMO Panel (2010b, 2011a). This includes, for each of the two treatments of maize DP23211, the application of a difference test (between the GM maize and its not treated comparator) and an equivalence test (between the GM maize and the set of not treated commercial reference varieties). The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).[11]

3.4.3 | Suitability of selected test materials

3.4.3.1 | *Selection of the test materials*

As described in Section 3.3.1, inbred line PHR03 was transformed to obtain line DP23211, which was then crossed with the non-GM inbred line PHEJW to produce the hybrid DP23211[12] used to conduct the agronomic and phenotypic and the compositional assessment.

The comparator used in the field trials is the non-GM maize hybrid PHEJW × PHR03, which is isogenic to hybrid maize DP23211 (as documented by the pedigree), and is considered to be the conventional counterpart.

Hybrid maize DP23211 and the conventional counterpart (PHEJW × PHR03), both with a comparative relative maturity (CRM) of 107, are appropriate for growing in a range of environments across North America, where the comparative field trials were conducted.

The non-GM reference varieties (see Table 2) with a CRM ranging from 106 to 111 were selected by the applicant and, at each selected site, four of them were tested. On the basis of the information provided on relative maturity classes, the GMO Panel considers the selected non-GM reference varieties appropriate for the comparative assessment.

3.4.3.2 | *Seed production and quality*

The seeds of maize DP23211 and of the conventional counterpart used in the 2018 field trials (see Table 2) were produced, harvested and stored under similar conditions. The seed lots were verified for their identity via event-specific PCR analysis. The germination of GM maize DP23211 and the conventional counterpart was tested under warm and cold temperature conditions.[13] Germination capacity of the GM maize DP23211 was compared with the one of its conventional counterpart. The results of these studies indicate that the seed germination of maize DP23211 was not different than that of its conventional counterpart.[14]

The GMO Panel considers that the starting seeds used as test material in the agronomic, phenotypic and compositional studies were of adequate quality.

3.4.3.3 | *Conclusion on suitability*

The GMO Panel is of the opinion that maize DP23211, the conventional counterpart and the non-GM reference hybrid were properly selected and are of adequate quality. Therefore, the test materials are considered suitable for the comparative analysis.

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

[12]For the agronomic, phenotypic and compositional analysis, hybrid maize DP23211 refers to the event obtained crossing inbred line DP23211 PHR03 with the inbred line PHEJW.

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

[14]GM hybrid maize showed a mean germination of 97% under both temperature conditions while the conventional counterpart showed a mean of 96% under both temperature conditions.

3.4.4 | Representativeness of the receiving environments

3.4.4.1 | *Selection of field trial sites*

The selected field trial sites were located in commercial maize-growing regions of the US and Canada. Climate and soil characteristics of the selected fields were diverse,[15] 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 seven of the selected sites.[16] However, due to the lack of major impacts on plant growth at these sites, the GMO Panel considers that the exceptional weather conditions did not invalidate the selection of the field trial sites for the comparative analyses.

3.4.4.3 | *Management practices*

The field trials included plots containing maize DP23211, plots with the conventional counterpart and plots with non-GM reference varieties, managed according to local agricultural practices. In addition, the field trials included plots containing the GM maize managed following the same agricultural practices, but conventional herbicides were replaced with a single application at growth stage BBCH 14–15 to glufosinate-containing herbicides.[17] The GMO Panel considers that the management practices, including sowing, harvesting and application of plant protection products, were appropriate for the field trials.

3.4.4.4 | *Conclusion on representativeness*

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

3.4.5 | Agronomic and phenotypic endpoints

3.4.5.1 | *Agronomic and phenotypic endpoints tested under field conditions*

Eleven agronomic and phenotypic endpoints[18] plus information on biotic and abiotic stressors were collected from the field trials (see Table 2). Two endpoints (ear count and dropped ears) had 50% or more of the data values at a uniform value and were not subjected to the statistical analysis.

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

- For maize DP23211 (not treated with the intended herbicide), the test of difference identified statistically significant differences with the conventional counterpart for early stand count, days to flowering, final stand count, plant height and yield. All the endpoints fell under equivalence category I or II.
- For maize DP23211 (treated with the intended herbicide), the test of difference identified statistically significant differences with the conventional counterpart for early stand count, days to flowering, plant height and yield. All the endpoints fell under equivalence category I or II.

3.4.6 | Compositional analysis

Maize DP23211 forage and grains harvested from the field trials (Table 2) were analysed for 81 different constituents (10 in forage and 71 in grains), including the key constituents recommended by the Organisation for Economic Co-operation and Development (OECD, 2002). The statistical analysis was not applied to 10 grain constituents because their concentration in more than half of the samples were below the limit of quantification.[19]

[15]Soil types of the field trials were clay loam, sandy clay loam, sandy loam, silty clay loam, loam, silt loam and clay; soil organic carbon ranged from 1.2% to 2.3%; pH ranged from 5.1 to 8.1; average temperatures and sum of precipitations during the usual crop growing season ranged respectively from 12.5°C to 22.0°C and from 424 mm to 899 mm.

[16]Wind was recorded at two field trials in Iowa and at one field trial in Illinois, Pennsylvania and Texas. Hail and wind were recorded at one field trial in Minnesota and Nebraska.

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

[18]Early stand count, days to flowering, plant height, lodging, final stand count, days to maturity, ear count, dropped ears, harvest grain moisture, yield and 100-kernel weight.

[19]These were: lauric acid (C12:0), myristic acid (C14:0), heptadecanoic acid (C17:0), heptadecenoic acid (C17:1), eicosadienoic acid (C20:2), erucic acid (C22:1), vitamin B2 (riboflavin), β-tocopherol, δ-tocopherol and furfural.

The test of difference and the test of equivalence could be applied to 71 constituents (10 in forage[20] and 61 in grains[21]), with the following results (Table 3):

- For maize DP23211 not treated with the intended herbicide, the test of difference identified statistically significant differences with respect to the conventional counterpart for 13 constituents (1 in forage and 12 in grains). The test of equivalence between maize DP23211 and the non-GM maize reference varieties indicated that all those constituents fell under equivalence category I or II.
- For maize DP23211 treated with the intended herbicide, statistically significant differences with the conventional counterpart were identified for 21 constituents (2 in forage and 19 in grains). All these constituents fell under equivalence category I or II except for histidine, phenylalanine, magnesium and phosphorus in grains, which fell under equivalence category III or IV, while the test of equivalence was not applied for folic acid in grains (Table 3).

TABLE 3 Summary of the outcome of the comparative analysis in grain and forage from maize DP23211.

		Test of difference[a]			
		Not treated[c]		Treated[c]	
		Not different	Significantly different	Not different	Significantly different
Test of equivalence[b]	Category I/II	40	13[d]	43	16[d]
	Category III/IV	17[e]	–	7[e]	4[f]
	Not categorised	1[g]	–	–	1[h]
	Total endpoints	71		71	

[a]Comparison between the GM maize and the conventional counterpart.

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

[c]Treated/not treated with glufosinate ammonium.

[d]Endpoints with significant differences between the GM maize and the conventional counterpart and falling in equivalence category I-II. For forage, not treated only: none; treated only: carbohydrates; both treated and not treated: calcium. For grains, not treated only: ash, methionine, palmitoleic acid (C16:1), oleic acid (18:1), linoleic acid (C18:2), behenic acid (C22:0), α-tocopherol and *p*-coumaric acid. Treated only: arginine, glycine, lysine, tyrosine, palmitic acid (C16:0), copper, β-carotene, thiamine, ferulic acid, phytic acid. Both treated and not treated: stearic acid (C18:0), arachidic acid (C20:0), eicosenoic acid (C20:1), pyridoxine.

[e]Endpoints with no significant differences between the GM maize and the conventional counterpart and falling in equivalence category III/IV. In forage, none. In grains, not treated only: cystine, histidine, phenylalanine, serine, threonine, valine, manganese, phytic acid, magnesium and phosphorus; treated only: none; both not treated and treated: crude protein, alanine, aspartic acid, glutamic acid, isoleucine, leucine and proline. Categorization of amino acids under category III/IV is probably linked to the presence of crude protein also in these categories. No further safety assessment is needed for any of the endpoints listed here as no statistically significant differences were observed compared to the conventional counterpart.

[f]Endpoints with significant differences between the GM maize and the conventional counterpart and falling in equivalence category III/IV. In forage: none. In grains, not treated only: none; treated only: histidine, phenylalanine, magnesium and phosphorus; both treated and not treated; none. Quantitative results are reported in Table 4.

[g]Endpoints that were not categorised for equivalence and for which no significant differences were identified between the GM maize and the conventional counterpart. In forage, none. In grains, treated only: none; not treated only: folic acid.

[h]Endpoints that were not categorised for equivalence and for which significant differences were identified between the GM maize and the conventional counterpart. In forage, none. In grains, treated only: folic acid; not treated only: none. Quantitative results are reported in Table 4.

The GMO Panel assessed all the significant differences between maize DP23211 and its conventional counterpart, taking into account the potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties. Quantitative results for the endpoints showing a significant difference between maize DP23211 and its conventional counterpart and not falling under equivalence category I/II are given in Table 4.

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

[21]Moisture, crude protein, crude fat, crude fibre, acid detergent fibre (ADF), neutral detergent fibre (NDF), total dietary fibre, ash, carbohydrates, alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, zinc, palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), alpha-linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C20:1), behenic acid (C22:0), lignoceric acid (C24:0), β-carotene, thiamine, niacin, pantothenic acid, pyridoxine, folic acid, α-tocopherol, γ-tocopherol, total tocopherols, *p*-coumaric acid, ferulic acid, inositol, phytic acid, raffinose and trypsin inhibitor.

TABLE 4 Quantitative results (estimated means and equivalence limits) for the compositional endpoints in maize DP-23211, which are further assessed based on the results of the statistical analysis.

		Maize DP-23211			Non-GM reference varieties	
	Endpoint	Not treated[a]	Treated[a]	Conventional counterpart	Mean	Equivalence limits
Grain	Histidine (% dw)	0.316	0.310*	0.317	0.275	0.248–0.303
	Phenylalanine (% dw)	0.571	0.557*	0.573	0.471	0.387–0.555
	Magnesium (% dw)	0.132	0.128*	0.132	0.111	0.0984–0.123
	Phosphorus (% dw)	0.357	0.347*	0.359	0.315	0.284–0.346
	Folic acid (mg/kg dw)	1.23	1.30*	1.14	1.20	–

Note: For maize DP-23211, significantly different values are marked with an asterisk, while the outcomes of the test of equivalence are differentiated by greyscale backgrounds. Light and dark grey backgrounds correspond to equivalence category III and IV, respectively. A white background is used when the test of equivalence is not applied. dw: dry weight; –: test of equivalence not applied because of the lack of variation among the non-GM reference varieties.

[a]Treated/not treated with glufosinate ammonium.

3.4.7 | Conclusion on the comparative assessment

Considering the selection of test materials, the field trial sites and the associated management practices, and the agronomic/phenotypic characterisation as an indicator of the overall field trial quality, the GMO Panel concludes that the field trials are appropriate to support the comparative analysis.

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

- None of the differences identified in agronomic and phenotypic characteristics between maize DP23211 and the conventional counterpart needs further assessment.
- None of the compositional differences identified between maize DP23211 and the conventional counterpart needs further assessment except for those in levels of histidine, phenylalanine, magnesium, phosphorus and folic acid in grain, which are further assessed in Section 3.5.

3.5 | Food/feed safety assessment[22]

3.5.1 | Effects of processing

Maize DP23211 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, 2017a, 2021a). 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). It has been shown, for example, that when characteristics of known food allergens are examined, a prominent trait 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 the newly expressed PAT and PMI proteins have been previously evaluated by the GMO Panel (EFSA GMO Panel, 2012, 2015b, 2017b). No new information has been provided in the context of this application.

Furthermore, the applicant provided an additional study on the effects of temperature on the IPD072Aa protein. Samples of IPD072Aa protein were incubated for 30–35 minutes at 25°C, 50°C, 60°C, 95°C or 121°C followed by western blot analysis or by a bioassay measuring protein activity. The study showed that the IPD072Aa protein was inactive when treated for 30 min at 121°C. In relation to the effect of pH on the IPD072Aa protein, the molecular mass and immunoreactivity of the protein was unchanged at pH 1.2 and 7.5.

[22]Dossier: Part II – Sections 1.4, 1.5, 1.6, 2, 3 and 4; additional information: 17/12/2020, 11/3/2021, 29/6/2021, 7/7/2021, 13/7/2021 and 10/11/2021.

3.5.2.2 | In vitro *protein degradation by proteolytic enzymes*

The resistance to degradation by pepsin of the newly expressed PAT and PMI proteins have been previously evaluated by the GMO Panel (EFSA GMO Panel, 2012, 2015b, 2017b). No new information has been provided in the context of this application.

Furthermore, the applicant provided an additional study where the resistance to degradation by pepsin of the IPD072Aa protein was investigated in solutions at pH ~1.2. 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 IPD072Aa protein was degraded by pepsin within 0.5 min of incubation.

3.5.3 | Toxicology

3.5.3.1 | *Testing of newly expressed proteins*

Three proteins (IPD072Aa, PAT and PMI) are newly expressed in maize DP23211.

The PAT and PMI proteins were extensively characterised and found to match the expected deduced amino acid sequences (see Section 3.3.3). The PAT and PMI proteins were previously assessed by the GMO Panel (EFSA GMO Panel, 2012, 2015b, 2017b) and no safety concerns for humans and animals were identified. Updated bioinformatics analysis did not reveal similarities of these proteins to known toxins. The GMO Panel is not aware of any new information that would change the previous conclusion of the risk assessment that these proteins do not raise safety concern.

The IPD072Aa protein has not been previously assessed by the GMO Panel. Information on the source organism and on the mode of action of the protein, bioinformatic, *in vitro* degradation (Section 3.5.2.2) and toxicological studies were considered to assess the safety of this protein for humans and animals.

Source organism. The source organism of the gene coding for the IPD072Aa protein (*Pseudomonas chlororaphis)* has been reported to protect plants by producing compounds that inhibit fungal growth (EFSA, 2017c; EFSA BIOHAZ Panel, 2015), insects and nematodes and it is used in agriculture (Anderson et al., 2018; Anderson & Kim, 2018). The GMO Panel considers this information not sufficient to support the history of consumption of the newly expressed protein.

Mode of action of the IPD072Aa protein. The IPD072Aa protein is intended to target and disrupt midgut epithelial cells in certain coleopteran species (Western Corn Rootworm, WCR) (Carlson et al., 2019). Naturally occurring as a dimer, once ingested it disassociates into monomers that bind to the midgut enterocytes by specific receptors 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 towards WCR among insects.

Bioinformatic studies. No significant similarities of IPD072Aa protein to toxins and allergens were identified (see Section 3.3.2).

Acute study. Oral administration of IPD072Aa protein to male and female mice (2000 mg/kg body weight [bw]) did not result in mortality or other evidence of acute oral toxicity.

28-Day repeated dose toxicity study. The applicant provided a 28-day toxicity study in mice on IPD072Aa conducted in accordance with OECD TG 407 (2008) and to the principles of Good Laboratory Practice.

Groups of Crl:CD-1 mice (10/sex per group), 7–8 weeks old at the start of dosing were administered diets containing, respectively, the test substance (IPD072Aa protein) at targeted nominal doses of 1000, 300 or 100 mg/kg bw per day (high, medium and low IPD072Aa protein groups); 1000 mg/kg bw per day of bovine serum albumin (BSA) (BSA control group) or a basal diet (control group). 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 (*E. coli*, Lot Number PRCH-4044) and contained about 95% IPD072Aa protein. The amino acid sequence analysis of the *E. coli*-produced IPD072Aa used in this 28-day toxicity study by mass spectrometry matched the deduced sequence as defined by the *ipd072Aa* gene. This protein had the expected molecular weight and immunoreactivity to IPD072Aa-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 assessed the deviations to the protocol reported in the study. These 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 their preparations were homogeneous and exhibited acceptable stability. Mean achieved doses of IPD072Aa were 0, 87, 276 and 903 mg/kg bw per day in males and 0, 107, 325 and 1055 mg/kg bw per day in females in the control, low-, medium- and high-dose groups respectively.

An appropriate range of statistical tests was performed on the results of the study. A detailed description of the methodology and of statistically significant findings identified in mice given diets containing IDP072Aa test substance (100, 300 and 1000 mg/kg bw per day of test substance) is reported in Appendix C.

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[23] 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 dietary incorporation level.

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

The GMO Panel concludes that no adverse effects were observed in this 28-day mouse toxicity study on *E. coli*-produced IPD072Aa protein, at doses up to 1000 mg/kg bw per day.

Overall conclusion of the IPD072Aa protein safety. Based on the above information on the source organism and on the mode of action of the protein, bioinformatic, in vitro degradation and toxicological studies, the GMO Panel did not identified indications of safety concerns for the IPD072Aa protein in humans and animals.

The GMO Panel considers that there are no indications that the newly expressed IPD072Aa, PAT and/or PMI proteins in maize DP23211 may raise concerns for toxicity. Furthermore, 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 DP23211.

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 grains and forage from maize DP23211, with the exception of the intended expression of DvSSJ1 double-stranded RNA (dsRNA) and derived siRNAs, designed to control coleopteran pests via RNAi. According to the applicant, the gene-silencing effects of DvSSJ1 dsRNA are mainly driven by ingestion of dsRNA from the plant and its processing into siRNAs by the insects.

NcRNAs are ubiquitous in a broad range of organisms used for food and feed and, hence, are normal constituents of human and animal diet. Dietary ncRNAs are generally rapidly denaturated, depurinated and degraded shortly after ingestion due to enzymes and conditions (e.g. pH) in the gastrointestinal tract lumen; in addition, the presence of barriers (e.g. mucus, cellular membranes) limits the cellular uptake of ncRNAs by gastrointestinal cells, and a rapid intracellular degradation of possibly uptaken ncRNA occurs (Dávalos et al., 2019). Due to the above, the amount of RNAs taken up and absorbed after oral ingestion is considered negligible in humans and farmed animals (mammals, birds and fish).

Moreover, the GMO Panel noted that the structure of DvSSJ1 dsRNA does not show specific chemical modifications that would increase its stability in plant and/or its stability and cellular uptake in the gastrointestinal tract following oral administration. Specifically, the applicant reported that DvSSJ1 dsRNA has a typical hairpin structure and does not contain any other structural modification aimed to increase stability.

Therefore, it is highly unlikely that the DvSSJ1 dsRNA and its derived siRNAs are able to exert any biological effects once ingested by humans, mammals, birds and fish. Taking into account all of the above, the GMO Panel considers that no toxicological studies are necessary.

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

No altered levels of food/feed constituents have been identified in grains and forage maize DP23211, except for histidine, phenylalanine, magnesium, phosphorus and folic acid. 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.

[23]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).

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

Based on the outcome of 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 DP23211. Therefore, animal feeding studies with food/feed derived from maize DP23211 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 DP23211.

In this study, pair-housed Crl:CD(SD) rats (16/sex per group; 2 rats/cage) were allocated to five groups using a randomised complete block design with eight replications per sex. Groups were fed diets containing ground maize DP23211 grains from plants treated with the intended herbicide (glufosinate-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 (P0928, P0993 and P1150) (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 good laboratory practice (GLP) with some minor deviations described in the study report, not impacting the study results and interpretation.

The stability of the test and control materials was not 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 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 DP23211 is reported in Appendix D.

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 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 dietary incorporation level.

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

The GMO Panel concludes that no adverse effects were observed in rats in this 90-day toxicity study given diets containing maize DP23211 (up to 50% incorporation rate).

3.5.4 | Allergenicity

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

[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).

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

A weight-of-evidence approach was followed, taking into account all of the information obtained on the newly expressed protein, as no single piece of information or experimental method yielded sufficient evidence to predict allergenicity (Codex Alimentarius, 2009; EFSA GMO Panel, 2011a, 2017b; Regulation (EU) No 503/2013).

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

Updated bioinformatic analyses of the amino acid sequences of the IPD072Aa, PAT and PMI proteins, using the criterion of 35% identity in a sliding window of 80 amino acids, revealed no significant similarities to known allergens. In addition, the applicant performed analyses searching for matches of eight contiguous identical amino acid sequences between these newly expressed proteins and known allergens, which confirmed the outcome of the previous bioinformatic analyses.

The studies on resistance to degradation of the IPD072Aa, PAT and PMI proteins by pepsin are described in Section 3.5.2.2. No indications pointing to safety concerns were identified. The GMO Panel has previously evaluated the safety of the PAT and PMI proteins in the context of other applications and no concerns for allergenicity were identified (e.g. EFSA GMO Panel, 2012, 2015b, 2017b). The GMO Panel is not aware of any new information that would change this conclusion.

In addition, the GMO Panel did not find an indication that the newly expressed proteins IPD072Aa, PAT and PMI at the levels expressed in maize DP23211 might be adjuvants.

Furthermore, the applicant also provided information on the safety of the IPD072Aa, PAT and PMI proteins regarding their potential to cause a celiac disease response. For this assessment, the applicant followed the principles described in the EFSA GMO Panel guidance document (EFSA GMO Panel, 2017b). The assessment of the IPD072Aa protein identified no perfect or relevant partial matches with known celiac disease peptide sequences. The assessment of the PAT and PMI proteins revealed partial matches containing the Q/E-X1-P-X2 motif. These partial matches were previously assessed by the EFSA GMO Panel (2021b, 2022b, 2023) and no safety concerns were identified.

The GMO Panel considers that there are no indications that the newly expressed IPD072Aa, PAT and/or PMI proteins in maize DP23211 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. However, maize is not considered a common allergenic food[25] (OECD, 2002). Therefore, the GMO Panel does not request experimental data to analyse the allergen repertoire of GM maize. In the context of this application and considering the data from the molecular characterisation, the compositional analysis and the assessment of the newly expressed proteins (see Sections 3.3, 3.4 and 3.5), the GMO Panel identifies no indications of potentially increased allergenicity of food and feed derived from maize DP23211 compared with that derived from the non-GM comparator 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 IPD072Aa, PAT and PMI proteins newly expressed in maize DP23211. Dietary exposure was estimated based on protein expression levels reported in this application for maize DP23211 treated with glufosinate ammonium, the current available consumption data and feed practices, the foods and feeds currently available in the market and the described processing conditions. For the purpose of estimating dietary exposure, the levels of newly expressed proteins in maize DP23211 grains, forage and pollen were derived from replicated field trials (four replicates from six locations, $n = 24$) in 2018 in the US and Canada (see Section 3.4.1). Table 1 in Section 3.3.4 shows the protein expression levels used to estimate both human and animal dietary exposure.

Additionally, the applicant estimated the levels of DvSSJ1 dsRNA in grains and forage and provided dietary exposure estimates in humans and animals.[26] However, the human and animal dietary exposure to DvSSJ1 dsRNA is considered negligible since these molecules are generally rapidly denaturated, depurinated and degraded shortly after ingestion (see Section 3.5.3.2).

Human dietary exposure

Chronic and acute dietary exposure to IPD072Aa, PAT and PMI proteins newly expressed in maize DP23211 were provided. The applicant followed the methodology described in the EFSA Statement 'Human dietary exposure assessment to newly

[25]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.

[26]Study reports PHI-2019-161 and PHI-2019-205.

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 populations (pregnant and lactating women). Since no specific consumption data were available on commodities containing, consisting of or obtained from maize DP23211 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).[27] Corn oil was excluded from the assessment since no proteins are expected to be present in the oil.

Mean protein expression values on fresh weight basis 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.[28] 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 54.7 µg/kg bw per day for PAT and PMI proteins, and 25.8 µg/kg bw per day for IPD072Aa. 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 20.3 µg/kg bw per day for PAT and PMI proteins, and 9.6 µg/kg bw per day for IPD072Aa. The main contributor to the exposure in the dietary survey with the highest estimates was sweet corn.

An ad hoc dietary exposure scenario was also provided for consumers of pollen supplements under the assumption that these supplements might be made of pollen from maize DP23211. Consumption data on pollen supplements are available for few consumers across different European countries.[29] The low number of consumers available adds uncertainty to the exposure estimations, which should be carefully interpreted, 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 0.001 µg/kg bw per day for IPD072Aa to 33.1 µg/kg bw per day for PAT, in the elderly population. Similarly, the highest chronic dietary exposure in average consumers would range from 0.0005 µg/kg bw per day for IPD072Aa to 21.8 µg/kg bw per day for PAT, in the very elderly population.

Animal dietary exposure

Dietary exposure to IPD072Aa, PAT and PMI proteins in maize DP23211 was estimated across different animal species as described below, assuming the consumption of maize products commonly entering the feed supply chain (i.e. maize grains and forage). A conservative scenario with 100% replacement of conventional maize products by maize DP23211 products was considered.

Mean levels (dry weight) of the newly expressed proteins in grains and forage from maize DP23211 treated with the intended herbicide used for animal dietary exposure are listed in Table 1 in Section 3.3.4.

The applicant estimated dietary exposure to IPD072Aa, PAT and PMI proteins in livestock (i.e. poultry, swine, cattle and sheep), based on estimates for body weights, daily feed intakes and inclusion rates (percentage) of maize grains and forage in diets/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 E.

3.5.6 | Nutritional assessment of GM food/feed

The intended traits of maize DP23211 are herbicide tolerance and control of certain coleopteran pests, with no intention to alter nutritional parameters. However, levels of histidine, phenylalanine, magnesium, phosphorus and folic acid in maize grains (treated) were significantly different from its conventional counterpart and showed a lack of equivalence with the set of non-GM reference varieties or could not be categorised (Section 3.4.6). The biological relevance of these compounds, the role of DP23211 maize as contributor to their total intake and the magnitude and direction of the observed changes were considered during the nutritional assessment.

3.5.6.1 | *Human nutrition*

Overall, maize protein is considered of low nutritional quality due to a poor balance of indispensable amino acids, in particular due to the low levels of lysine and tryptophan. Although both histidine and phenylalanine are indispensable amino

[27] https://www.efsa.europa.eu/en/applications/gmo/tools. Data accessed: August 2019.

[28] 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 3.3 µg of PAT/g of maize bread as compared to the 3.6 µg/g reported as mean concentration in the maize grains (see Table 1, Section 3.3.4.).

[29] https://www.efsa.europa.eu/en/food-consumption/comprehensive-database. Data accessed: February 2021.

acids (EFSA NDA Panel, 2012), the relatively small decrease in treated maize DP23211 (~2% for histidine and ~3% for phenylalanine) as compared to its conventional counterpart does not raise any nutritional concern for maize DP23211, also considering the presence of these amino acids in all dietary proteins.

An increase of 14% of folic acid (folate, the natural form present in food) was observed in maize DP23211 (treated) as compared to its conventional counterpart. Green vegetables and certain (citrus) fruits are important dietary sources of folates. Although Tolerable Upper Intake Levels (UL) are set for folic acid, the relatively high upper limits (200–1000 μg/day[30]) as compared to the levels present in maize (~5 μg/100 g dw) make the observed changes irrelevant from a nutritional point of view.

Magnesium and phosphorus are the most abundant minerals in maize together with potassium. Phosphorus and magnesium are essential micronutrients for humans; while phosphorus is involved in many physiological processes (e.g. cell's energy cycle, cell regulation and signalling, etc.), magnesium is a cofactor of many enzymatic reactions that makes it essential in the intermediary metabolism for the synthesis of carbohydrates, lipids, nucleic acids and proteins (EFSA NDA Panel, 2015a, 2015b). Although maize as other grains are foods rich in magnesium and phosphorus, the presence of phytic acid drastically reduces the bioavailability of these micronutrients in maize (Gupta et al., 2015). Both minerals are widely distributed across many different foods: in the case of phosphorus, typically in foods high in protein content, i.e. milk and milk products followed by meat, poultry and fish, grain products and legumes; in the case of magnesium, apart from whole grains and grain products, fish and seafood, several vegetables, legumes, berries, bananas, and some coffee and cocoa beverage preparations (EFSA, 2017d). Considering this information, the ~3% decrease observed in the levels of phosphorus and magnesium in maize DP23211 (treated) as compared to its conventional counterpart is not considered of nutritionally concern.

3.5.6.2 | *Animal nutrition*

Histidine and phenylalanine are essential amino acids. Maize grains are not considered a major source of amino acids in animals, and the decrease of these amino acids in treated GM maize compared to the conventional counterpart is not a problem for animal nutrition because other protein sources can be used, in balanced diets, to satisfy the amino acids requirements, especially for monogastric animals.

Magnesium and phosphorus are major minerals; they are usually supplemented in diets because the amount, and the availability, of minerals present in feed could be not sufficient to satisfy the requirements. The observed decrease in treated GM maize compared to the conventional counterpart does not pose an issue for animals.

Folic acid is an important nutrient especially during lactation and pregnancy. Animals are not always able to synthesise it in sufficient amounts to meet physiological demands, especially monogastric animals, and the folate content of feed is rarely analysed (Ragaller et al., 2009). For these reasons, it is supplemented to the diet when needed. This vitamin is usually considered a nontoxic element. The oral administration of high dosage of folic acid is not considered dangerous to animals (National Research Council, 1987). Therefore, the observed increase observed in treated GM maize compared to the conventional counterpart is not a problem for animal nutrition.

3.5.7 | Post-market monitoring of GM food/feed

Maize DP23211, 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 IPD072Aa, PAT, PMI and the DvSSJ1 dsRNA and derived siRNAs newly expressed in maize DP23211 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 DP23211. The GMO Panel finds no evidence that the genetic modification impacts the overall safety of maize DP23211. Based on the outcome of the comparative assessment and the nutritional assessment, the GMO Panel concludes that the consumption of maize DP23211 does not represent any nutritional concern, in the context of the scope of this application. The GMO Panel concludes that maize DP23211, 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.

[30]Tolerable upper intake levels (UL) refer to folic acid, the synthetic form typically used as supplement and with higher bioavailability than food folate (0.5 μg of a folic acid supplement taken on an empty stomach = 1 μg food folate).

3.6 | Environmental risk assessment and monitoring plan[31]

Considering the scope of application EFSA-GMO-NL-2019-163, which excludes cultivation, the environmental risk assessment (ERA) of maize DP23211 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 DP23211 grains, during transportation and/or processing (EFSA GMO Panel, 2010a).

3.6.1 | Environmental risk assessment

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

It is unlikely that the intended traits of maize DP23211 will provide a selective advantage to maize plants, except when they are exposed to glufosinate-containing herbicides or infested by insect pests that are susceptible to DvSSJ1 dsRNA or to the IPD072Aa protein. However, this fitness advantage will not allow the GM plant to overcome other biological and abiotic factors (described above) limiting the 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 DP23211 will differ from conventional maize hybrid varieties in its ability to survive until subsequent seasons, or to establish occasional feral plants under European environmental conditions in case of accidental release into the environment of viable maize DP23211 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 HGT of DNA, or through vertical gene flow via cross-pollination from feral plants originating from spilled grains.

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

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

Homologous recombination is the only mechanism 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 DP23211 revealed that the genetic elements encoding for PAT and IPD072Aa proteins were codon-optimised and did not provide sufficient sequence identity to bacterial DNA. Alignments were 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.

In summary, there is no indication for an increased likelihood of horizontal transfer of DNA from maize DP23211 to bacteria. Given the nature of the recombinant DNA, the GMO Panel identified no safety concern linked to an unlikely but theoretically possible HGT.

[31]Dossier: Part II – Section 5 and 6; additional information: 11/3/2021.

Plant-to-plant gene transfer. The potential for occasional feral maize DP23211 plants originating from grain import spills to transfer recombinant DNA to sexually compatible plants and the environmental consequences of this transfer were considered.

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

Maize is an annual predominantly cross-pollinating crop. Cross-fertilisation occurs mainly by wind (OECD, 2003). Vertical gene transfer from maize is limited to *Zea* species. Wild relatives of maize outside cultivation are not known/reported in Europe (Eastham & Sweet, 2002; EFSA, 2016, 2022; OECD, 2003; Trtikova et al., 2017). Therefore, potential vertical gene transfer is restricted to maize and weedy *Zea* species, such as teosintes, and/or maize-teosinte hybrids, occurring in cultivated areas (EFSA, 2016, 2022; Le Corre et al., 2020; Trtikova et al., 2017).

The potential of spilled maize grains to establish, grow and produce pollen is extremely low and transient (see Section 3.6.1.1). Therefore, the likelihood/frequency of cross-pollination between occasional feral GM maize plants resulting from grain spillage, and weedy or cultivated *Zea* plants is considered extremely low (EFSA, 2016, 2022). Even if cross-pollination would occur, the GMO Panel is of the opinion that environmental effects as a consequence of the spread of genes from occasional feral GM maize plants in Europe will not differ from that of conventional maize varieties for the reasons given in Section 3.6.1.1.

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

Taking the scope of application EFSA-GMO-NL-2019-163 (no cultivation), potential interactions of occasional feral maize DP23211 plants arising from grain import spills with 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 DP23211 grains is limited, and because ingested dsRNA and 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 DP23211 with non-target organisms do not raise any environmental safety concern. Interactions that may occur between the insecticidal protein IPD072Aa and dsRNA will not alter this conclusion.

3.6.1.5 | *Interactions with the abiotic environment and biogeochemical cycles*

Given that environmental exposure to spilled GM material or occasional feral maize DP23211 plants arising from grain import spills is limited, and because ingested dsRNA and proteins are degraded before entering the environment through faecal material of animals fed with GM maize, the GMO Panel considers that potential interactions with the abiotic environment and biogeochemical cycles do not raise any environmental safety concern.

3.7 | 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 DP23211, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for maize DP23211 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 DP23211. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

3.7.1 | Conclusion of the environmental risk assessment and monitoring plan

The GMO Panel concludes that it is unlikely that maize DP23211 would differ from conventional maize varieties in its ability to persist under European environmental conditions. Considering the scope of application EFSA-GMO-NL-2019-163, interactions of occasional feral maize DP23211 plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from maize DP23211 to bacteria does not indicate a safety concern. Therefore, considering the

introduced traits, the outcome of the comparative analysis, and the routes and levels of exposure, the GMO Panel concludes that maize DP23211 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 DP23211.

4 | OVERALL CONCLUSIONS

The GMO Panel was asked to carry out a scientific assessment of maize DP23211 for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003. The molecular characterisation data establish that maize DP23211 contains a single insert consisting of one copy of the *pmi*, *mo-pat*, and *ipd072Aa* and DvSSJ1 dsRNA expression cassettes. The quality of the sequencing methodology and datasets was assessed by the EFSA GMO Panel and is in compliance to the requirements listed in the EFSA Technical Note. Updated bioinformatics analyses of the sequences encoding the newly expressed protein and other ORFs present within the insert or spanning the junctions between the insert and genomic DNA, do not raise any safety concerns. The *in planta* RNAi off-target search, performed with the sequence of the DvSSJ1 dsRNA, does not provide indication for an off-target effect that would need further safety assessment. The stability of the inserted DNA and of the introduced trait was confirmed over several generations.

The methodology used to quantify the levels of the IPD072Aa, PAT and PMI proteins is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of plant- and microbe-derived IPD072Aa proteins, indicate that these proteins are equivalent, and the microbe-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 DP23211 and its conventional counterpart needs further assessment, except for those in levels of histidine, phenylalanine, magnesium, phosphorus and folic acid in grain, which does not raise safety and nutritional concerns. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the IPD072Aa, PAT and PMI proteins and the DvSSJ1 dsRNA and derived siRNAs as expressed in maize DP23211. The GMO Panel finds no evidence that the genetic modification impacts on the overall safety of maize DP23211. In the context of this application, the consumption of food and feed from maize DP23211 does not represent a nutritional concern in humans and animals. Furthermore, no post-market monitoring of food/feed is considered necessary.

The GMO Panel concludes that there is a very low likelihood of environmental effects resulting from the accidental release of viable grains from maize DP23211 into the environment. The PMEM plan and reporting intervals are in line with the intended uses of maize DP23211. Based on the relevant publication identified through the literature searches, the GMO Panel did not identify any safety issues pertaining to the intended uses of maize DP23211.

The GMO Panel concludes that maize DP23211 is as safe as its conventional counterpart and the tested non-GM maize reference varieties with respect to potential effects on human and animal health and the environment.

5 | DOCUMENTATION AS PROVIDED TO EFSA

- Letter from the Competent Authority of the Netherlands received on 13th December 2019 concerning a request for authorization of the placing on the market of genetically modified maize DP23211 submitted in accordance with Regulation (EC) No 1829/2003 by Pioneer Hi-Bred International, Inc. as represented by Pioneer Overseas Corporation (EFSA Ref. EFSA-GMO-NL-2019-163; EFSA-Q-2019-00807).
- The application was made valid on 16th April 2020.
- Additional Information (Clock 1) was requested on 17th April 2020.
- Additional Information (Clock 1) was received on 8th May 2020.
- Additional Information (Clock 2) was requested on 16th June 2020.
- Additional Information (Clock 2) was received on 9th October 2020.
- Additional Information (Clock 3) was requested on 22nd October 2020.
- Additional Information (Clock 3) was received on 17th December 2020.
- Additional Information (Clock 4) was requested on 21st December 2020.
- Additional Information (Clock 4) was received on 11th March 2021 partial; 29th June 2021 complete.
- Additional Information (Clock 5) was requested on 25th February 2021.
- Additional Information (Clock 5) was received on 13th June 2023.
- Additional Information (Clock 6) was requested on 10th May 2021.
- Additional Information (Clock 6) was received on 7th July 2021 partial; 13th July 2021 complete.
- Additional Information (Clock 7) was requested on 15th October 2021.
- Additional Information (Clock 7) was received on 10th November 2021.
- Additional Information (Clock 8) was requested on 9th December 2021.
- Additional Information (Clock 8) was received on 20th December 2021.

- Additional Information (Clock 9) was requested on 15th July 2022.
- Additional Information (Clock 9) was received on 26th January 2023.
- Supplementary information was provided on voluntary basis on 8th May 2023.
- Additional Information (Clock 10) was requested on 22nd June 2023.
- Additional Information (Clock 10) was received on 22 August 2023 partial; 15th September 2023 complete.
- Additional Information (Clock 11) was requested on 25th September 2023.
- Additional Information (Clock 11) was received on 8th November 2023.

ABBREVIATIONS

ADF	acid detergent fibre
bp	base pai
bw	body weight
CaMV	cauliflower mosaic virus genome
CRM	comparative relative maturity
DsRed2	red fluorescent gene from *Discosoma* sp.
dsRNA	double-stranded ribonucleic acid
dw	dry weight
ELISA	enzyme-linked immunosorbent assay
ERA	environmental risk assessment
Flp	flippase gene
fw	fresh weight
GLP	good laboratory practice
GMO	genetically modified organism
HGT	horizontal gene transfer
HR	homologous recombination
JSA	junction sequence analysis
Ltp2	lipid transfer protein gene
MS	mass spectrometry
NGS	next generation sequencing
NDF	neutral detergent fibre
odp2	ovule development protein 2
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
os-actin	actin gene from *Oryza sativa*
PAT	phosphinothricin acetyltransferase
PCR	polymerase chain reaction
pinII	proteinase inhibitor II gene
PMEM	post-market environmental monitoring
PMI	phosphomannose isomerase
qPCR	quantitative polymerase chain reaction
sbubi	ubiquitin gene from *Sorghum bicolor*
sb-gkaf	γ-kafarin gene from *Sorghum bicolor*
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
SSI	site-specific integration
st-LS1	LS1 gene of *Solanum tuberosum*
TDI	total daily intake
T-DNA	transfer-deoxyribonucleic acid
TEV	tobacco etch virus
ubiZM1	ubiquitin gene 1 of *Zea mays*
UBQ14	ubiquitin 14 gene
UL	Tolerable Upper Intake Level
UTR	untranslated region
WCR	western corn rootworm
wus2	Wuschel2 gene

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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.

REQUESTOR

Competent Authority of The Netherlands

QUESTION NUMBER

EFSA-Q-2019-00807

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SUPPORTING INFORMATION

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

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APPENDIX A

Additional studies

List of additional studies performed by or on behalf of the applicant with regard to the evaluation of the safety of maize DP23211 for humans, animal or the environment.

Study identification	Title
PHI-2018-039/701	Evaluation of Seed Germination, Health, and Viability of DP-Ø23211-2 Maize Seed Lots Identified for Use in Regulatory Science Studies
PHI-2018-193	Evaluation of Germination and Viability of Maize Line Containing Event DP-Ø23211-2
PHI-2018-215	An 8-week channel catfish (*Ictalurus punctatus*) dietary tolerance study of maize grain containing event DP-Ø23211-2
PHI-2019-001	Nutritional Equivalency Study of Maize Grain DP-Ø23211-2: Poultry Feeding Study
PHI-R056-Y18	Comparison of the DvSSJ1 Fragment to the Human Transcriptome
PHI-R046-Y19	Comparison of the DvSSJ1 Fragment to a Variety of Farm and Companion Ani-mal Transcriptomes

APPENDIX B

List of relevant publications identified by the applicant through literature searches (2009–June 2023)

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APPENDIX C

Statistical analysis and statistically significant findings in the 28-day toxicity study in mice on *E. coli* produced IPD072Aa protein

C1 Statistical analysis of the 28-day study on *E. coli* produced IPD072Aa 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 IPD072Aa protein groups) separately with the two control groups (basal and BSA), and the two control groups with each other. The analysis was performed for male and female mice separately. Continuous endpoints were analysed with a linear model (factor: diet group); for endpoints measured on a discrete scale, the comparisons were performed with Wilcoxon rank-sum test or with Fisher's exact test as appropriate. 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 E. coli produced IPD072Aa protein in mice.

Statistically significant parameter/endpoint	Finding	GMO Panel interpretation
Mean body weight gains and mean food efficiency	Higher in low- and intermediate-dose group males from Days 7 to 14. Lower in low- and intermediate group females from Days 7 to 14.	Sporadic findings with no dose response (not seen at the top dose) and with no impact on the terminal body weights. Not an adverse effect of treatment.
Mean cumulative body weight gain	Lower in the IPD072Aa low main study group females from Days 0 to 14.	Sporadic finding with no dose response (not seen at higher doses) and with no impact on the terminal body weights or cumulative gain Days 0–28. Not an adverse effect of treatment.
FOB and Motor activity		
Faecal pellet presence	Increased in top-dose males.	Within normal variation. Not an adverse effect of treatment.
Unusual posture	Increased in mid-dose females.	Within normal variation. No dose response. Not an adverse effect of treatment.
Ambulatory counts	Reduced (30%) in mid- and high-dose males at minutes 11–20.	Within normal variation. No dose response. No significant change in total ambulatory counts. Not an adverse effect of treatment.
Haematology		
Mean haematocrit	Lower (10%) in females in low-dose group (compared to basal control group).	Small magnitude. No dose response. Not an adverse effect of treatment.
Mean red blood cell corpuscular volume	Lower (10%) in females in low-dose group (compared to basal control group).	Small magnitude. No dose response. Not an adverse effect of treatment.
Mean absolute monocyte counts	Higher (2.5-fold) in high-dose group females (compared to basal control group).	Within normal variation. No change in the overall white blood cell distribution. Not an adverse effect of treatment.
Liver histopathology	Altered pattern of infiltrating cells in top-dose males; increased mononuclear cell infiltration in top-dose females.	A frequent finding in mouse livers. No other changes in liver histopathology, organ weight or clinical chemistry markers of liver function/damage. No increases in infiltration of other organs. Not an adverse effect of treatment.

APPENDIX D

Statistical analysis and statistically significant findings in the 90-day toxicity study in rats on maize DP23211

D1 Statistical analysis of the 90-day study on maize DP23211 in rats.

The following endpoints were statistically analysed: body weight, body weight gain, feed consumption and feed efficiency, forelimb and hindlimb strength, motor activity data, haematology, coagulation, hormone, urinalysis and clinical chemistry values, absolute and relative organ weights and functional observation battery (FOB) findings. For all continuous endpoints, mean and standard deviation were provided for each dose group for each sex, variable and period or time interval. In the statistical analysis, rats consuming the low- and high-dose test diets (at 17% and 30% inclusion level, respectively) were compared with those consuming the control diet. The data for the three reference groups were not included in the analysis. For continuous parameters, a linear mixed model was applied to data for individual animals for the two sexes combined (fixed effects: diet, sex and sex-by-diet interaction; random effects: block-within-sex and cage). Test-control comparisons were done both across sexes and separately for males and females; in case a significant sex-by-diet interaction was identified, only the sex-specific results were considered for the assessment. The model for continuous data was modified as needed for the analysis of sex-specific endpoints and cage-level data (food consumption and food efficiency). For each comparison, point estimates and 95% confidence intervals of the SES were reported to aid the assessment. For endpoints measured on a discrete scale, the comparisons were performed with Wilcoxon rank-sum test or with Fisher's exact test as appropriate. The data for the three reference groups, together with appropriate historical control data, were used to calculate a reference range of variability of the parameters to support the assessment of statistically significant results. Missing data were considered by the Panel and found not to affect the results.

TABLE D.1 Statistically significant findings in 90-day study on maize DP23211 in rats.

Statistically significant parameter/endpoint	Finding	GMO panel interpretation
Body weight and body weight gain	Reduced in top-dose females during most of the study.	Low magnitude with less than 5% deficit in absolute body weight at day 90. Not an adverse effect of treatment.
Food consumption	Increased (8%) in top dose animals Days 51–57.	Sporadic change with no significant finding over the entire study (2.3% increase). Low magnitude. Not an adverse effect of treatment.
Food efficiency	Reduced (5%–12%) in top-dose animals over the study.	Low magnitude. Within normal variation and no significant impact on terminal body weight. Not an adverse effect of treatment.
Motor activity	Increased (up to 100%) in top-dose females in periods 2, 3 and overall.	One control female had unusually low scores. Within normal variation. Not an adverse effect of treatment.
Serum alkaline phosphatase activity	Increased (13%–23%) in low-dose animals.	Low magnitude and within normal variation. No dose response and no associated histopathology findings. Not an adverse effect of treatment.
Serum triglycerides	Decreased (33%) in top-dose males.	One control male had an unusually high value. No equivalent change in females. Within normal variation Not adverse in isolation and no changes in related parameters. Not an adverse effect of treatment.
Thyroid weight (absolute, relative to body weight and brain weight)	Decreased (10%–15%) in low-dose females.	Small magnitude. No dose response. Within normal variation. No associated changes in pathology or thyroid hormone levels. Not an adverse effect of treatment.

APPENDIX E

Animal dietary exposure

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

			IR (%)		IPD072Aa		
	BW (kg)	TDI feed (kg DM/animal)	Grain (G)	Forage (F)	G	F	G+F
Beef cattle[a]	500	12	80	80	0.042	0.42	0.46
Dairy cattle	650	25	30	60	0.025	0.51	0.53
Ram/ewe	75	2.5	30	NA	0.022	NA	NA
Lamb	40	1.7	30	30	0.028	0.28	0.31
Breeding pigs	260	6	70	20	0.036	0.10	0.14
Finishing pigs	100	3	70	NA	0.046	NA	NA
Broiler	1.7	0.12	70	NA	0.11	NA	NA
Layer	1.9	0.13	70	10	0.11	0.15	0.26
Turkey	7	0.50	50	NA	0.079	NA	NA
			IR (%)		PAT		
	BW (kg)	TDI feed (kg DM/animal)	Grain (G)	Forage (F)	G	F	G+F
Beef cattle[a]	500	12	80	80	0.088	0.19	0.28
Dairy cattle	650	25	30	60	0.053	0.23	0.28
Ram/ewe	75	2.5	30	NA	0.046	NA	NA
Lamb	40	1.7	30	30	0.059	0.13	0.18
Breeding pigs	260	6	70	20	0.074	0.046	0.12
Finishing pigs	100	3	70	NA	0.097	NA	NA
Broiler	1.7	0.12	70	NA	0.23	NA	NA
Layer	1.9	0.13	70	10	0.22	0.068	0.29
Turkey	7	0.50	50	NA	0.16	NA	NA
			IR (%)		PMI		
	BW (kg)	TDI feed (kg DM/animal)	Grain (G)	Forage (F)	G	F	G+F
Beef cattle[a]	500	12	80	80	0.088	0.25	0.34
Dairy cattle	650	25	30	60	0.053	0.30	0.35
Ram/ewe	75	2.5	30	NA	0.046	NA	NA
Lamb	40	1.7	30	30	0.059	0.17	0.22
Breeding pigs	260	6	70	20	0.074	0.06	0.13
Finishing pigs	100	3	70	NA	0.097	NA	NA
Broiler	1.7	0.12	70	NA	0.23	NA	NA
Layer	1.9	0.13	70	10	0.22	0.089	0.31
Turkey	7	0.50	50	NA	0.16	NA	NA

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.