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SCIENTIFIC OPINION

Assessment of genetically modified maize MON 95275 (application GMFF-2022-5890)

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Abstract

Genetically modified maize MON 95275 was developed to confer protection to certain coleopteran species. These properties were achieved by introducing the *mpp75Aa1.1*, *vpb4Da2* and *DvSnf7* expression cassettes. The molecular characterisation data and bioinformatic analyses reveal similarity to known toxins, which was further assessed. None of the identified differences in the agronomic/phenotypic and compositional characteristics tested between maize MON 95275 and its conventional counterpart needs further assessment. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the Mpp75Aa1.1 and Vpb4Da2 proteins and the DvSnf7 dsRNA and derived siRNAs as expressed in maize MON 95275 and finds no evidence that the genetic modification would change the overall allergenicity of maize MON 95275. In the context of this application, the consumption of food and feed from maize MON 95275 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that maize MON 95275 is as safe as the conventional counterpart and non-GM maize varieties tested, and no post-market monitoring of food/feed is considered necessary. In the case of accidental release of maize MON 95275 material into the environment, this would not raise environmental safety concerns. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of maize MON 95275. The GMO Panel concludes that maize MON 95275 is as safe as its conventional counterpart and the tested non-GM maize varieties with respect to potential effects on human and animal health and the environment.

KEYWORDS

DvSnf7, genetic engineering, GM, import and processing, maize (*Zea mays*), MON 95275, Mpp75Aa1.1, Vpb4Da2

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SUMMARY

Following the submission of application GMFF-2022-5890 EFSA-Q-2022-00330 under Regulation (EC) No 1829/2003 from Bayer CropScience LP (referred to hereafter as 'the applicant'), the Panel on genetically modified organisms of the European Food Safety Authority (referred to hereafter as 'GMO Panel') was asked to deliver a Scientific Opinion on the safety of genetically modified (GM) insect-protected maize (*Zea mays* L.) MON 95275 according to Regulation (EU) No 503/2013. The scope of application EFSA-Q-2022-00330 is for import, processing and food and feed uses within the European Union (EU) of maize MON 95275 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 MON 95275 according to the scope of the application EFSA-Q-2022-00330. The GMO Panel conducted the assessment of maize MON 95275 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 MON 95275 contains a single insert consisting of one copy of the *mpp75Aa1.1*, *vpb4Da2* and *DvSnf7* dsRNA expression cassettes. The quality of the sequencing methodology and data sets was assessed by the EFSA GMO Panel and is in compliance with 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 reveal significant similarity to known toxins, which was further assessed. The in planta RNAi off-target search, performed with the sequence of the *DvSnf7* 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 Mpp75Aa1.1 and Vpb4Da2 proteins is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of plant and microbe-produced Mpp75Aa1.1 and Vpb4Da2 proteins indicate that these proteins are equivalent, and the microbial derived proteins can be used in the safety studies.

Considering the selection of test materials, the field trial sites and the associated management practices and the agronomic–phenotypic characterisation as an indicator of the overall field trial quality, the GMO Panel concludes that the field trials are appropriate to support the comparative analysis. None of the identified differences in the agronomic/phenotypic and compositional characteristics tested between maize MON 95275 and its conventional counterpart needs further assessment. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the Mpp75Aa1.1 and Vpb4Da2 proteins and the *DvSnf* 7 dsRNA and derived siRNAs as expressed in maize MON 95275 and finds no evidence that the genetic modification would change the overall allergenicity of maize MON 95275. In the context of this application, the consumption of food and feed from maize MON 95275 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that maize MON 95275 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 MON 95275 would not raise safety concerns in the case of accidental release of GM maize material into the environment. The post-market environmental monitoring (PMEM) plan and reporting intervals are in line with the intended uses of maize MON 95275.

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 MON 95275.

The GMO Panel concludes that maize MON 95275 is as safe as its conventional counterpart and the tested non-GM maize reference varieties with respect to potential effects on human and animal health and the environment.

1 | INTRODUCTION

The scope of the application GMFF-2022-5890 EFSA-Q-2022-00330 is for food and feed uses, import and processing of maize MON 95275 and does not include cultivation in the European Union (EU). Maize MON 95275 was developed to confer control of certain coleopteran pests.

1.1 | Background

On 23 May 2022, the European Food Safety Authority (EFSA) received from the Competent Authority of The Netherlands application EFSA-Q-2022-00330 for authorisation of maize MON 95275 (Unique Identifier MON-95275-7), submitted by Bayer CropScience LP (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003.[1] Following receipt of application EFSA-Q-2022-00330, EFSA informed EU Member States (MS) and the European Commission, and made the application available to them. Simultaneously, EFSA published a 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 29 August 2022, EFSA declared the application valid.

From validity date, EFSA and the Panel on genetically modified organisms of the European Food Safety Authority (referred to hereafter as 'GMO Panel') endeavoured to respect a time limit of 6 months to issue a scientific opinion on application GMFF-2022-5890. Such time limit was extended whenever EFSA and/or GMO Panel requested supplementary information to the applicant. According to Regulation (EC) No 1829/2003, any supplementary information provided by the applicant during the risk assessment was made available to the EU Member States and European Commission (for further details, see the section 'Documentation', below). In accordance with Regulation (EC) No 1829/2003, EFSA consulted the nominated risk assessment bodies of EU Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC.[4] The EU Member States had 3 months to make their opinion known on application GMFF-2022-5890 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 MON 95275 in the context of its scope as defined in application GMFF-2022-5890.

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

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a confidential and a non-confidential version of the dossier GMFF-2022-5890 following the EFSA requirements as detailed in EFSA (2021a, 2021b).

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

According to Art. 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations,[7] EFSA carried out a public consultation on the non-confidential version of the dossier from 13 January to 03 February 2023 for which no comments were received.

The GMO Panel based its scientific assessment of maize MON 95275 on the valid application GMFF-2022-5890 EFSA-Q-2022-00330, additional information provided by the applicant during the risk assessment, relevant scientific comments submitted by EU MS and relevant peer-reviewed scientific publications. As part of this comprehensive information package,

[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]https://open.efsa.europa.eu/dossier/GMFF-2022-5890?type=node&key=238382.
[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]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–48.
[6]https://open.efsa.europa.eu/questions/EFSA-Q-2022-00330.
[7]Decision https://www.efsa.europa.eu/sites/default/files/corporate_publications/files/210111-PAs-pre-submission-phase-and-public-consultations.pdf.

the GMO Panel received an additional unpublished study submitted by the applicant in order to comply with the specific provisions of Regulation (EU) No 503/2013. This additional unpublished study is provided in Appendix A.

2.2 | Methodologies

The GMO Panel conducted its assessment in line with the principles described in Regulation (EU) No 1829/2003, the applicable guidelines (i.e. EFSA GMO Panel, 2010a, 2011a, 2011b, 2015; EFSA Scientific Committee, 2011) and explanatory notes and statements (i.e. EFSA GMO Panel, 2010b, 2018a, 2021; EFSA, 2010, 2014, 2017, 2018, 2019a, 2019b, 2021a, 2021b) for the risk assessment of GM plants.

For this application, in the context of the contracts OC/EFSA/GMO/2018/04, OC/EFSA/GMO/2020/01, OC/EFSA/GMO/2021/06, the contractors performed preparatory work for the evaluation of the applicant's literature search, the completeness and quality of DNA sequencing information, bioinformatic analyses on maize MON 95275, respectively.

3 | ASSESSMENT

3.1 | Introduction

Maize MON 95275 expresses two insecticidal proteins Mpp75Aa1.1 and Vpb4Da2 which are members of the pore-forming ETX_MTX2 β-PFPs protein family and of the Bacterial_exotoxin_B protein family, respectively, and the DvSnf7.1 double-stranded ribonucleic acid (dsRNA). The insecticidal proteins and the dsRNA provide control for coleopteran pests including the northern corn rootworm (*Diabrotica barberi*) and the western corn rootworm (*Diabrotica virgifera virgifera*).

3.2 | Systematic literature review[8]

The GMO Panel assessed the applicant's literature searches on maize MON 95275, which include a scoping review, according to the guidelines given in EFSA (2010, 2019b).

A systematic review as referred to in Regulation (EU) No 503/2013 has not been provided in support to the risk assessment of application GMFF-2022-5890. Based on the outcome of the scoping review, the GMO Panel agrees that there is limited value of undertaking a systematic review for maize MON 95275 at present.

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

The literature searches identified five relevant peer-reviewed and non-peer-reviewed publications on maize MON 95275 (Appendix B). Based on the relevant publications, the GMO Panel does not identify any safety issues pertaining to the intended uses of maize MON 95275.

3.3 | Molecular characterisation[9]

3.3.1 | Transformation process and vector constructs

Maize MON 95275 was developed by a two-step process. In the first step, immature embryos of maize inbred line LH244 were co-cultured with a disarmed *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*) strain ABI containing the vector PV-ZMIR525664. In the second step, selected R2 lines were crossed with a maize HCL617 line expressing Cre recombinase, which had been transformed with vector PV-ZMOO513642. In the resulting plants, the CP4 EPSPS cassette was excised by the Cre recombinase, and the *Cre* gene was subsequently segregated away, through conventional breeding, to obtain MON 95275.

The plasmid PV-ZMIR525664 used for the transformation of LH244 carries three expression cassettes between the right and left border of the T-DNA, containing the following genetic elements:

- The *DvSnf7*.1 suppression cassette consisting of the optimised *pIIG-Zm1* enhancer from the *physical impedance induced protein (pIIg)* gene from *Zea mays*, the 35S promoter and leader sequence of the 35S RNA of cauliflower mosaic virus (CaMV), the *Hsp70* intron and flanking exon sequence of the *hsp70* gene from *Zea mays*, the coding region to express two fragments of inverted RNA repeats of the *Snf7* gene from *Diabrotica virgifera*, the 3′ UTR sequence of the *E9* gene from the *rbcS* gene family of *Pisum sativum*.
- The *mpp75Aa1.1* expression cassette consisting of *DaMv-1* enhancer from *Dalia mosaic virus* (DaMV), the *RCc3-Td1* promoter and leader sequence of the *RCc3* gene from *Tripsacum dactyloides*, the intron of a putative *14-3-3c* gene from

[8]Dossier: Part II – Section 7; additional information: 17/02/2023, 12/05/2023, 15/12/2023 and 03/06/2024.
[9]Dossier: Part II – Section 1.2; additional information: 07/11/2022, 17/02/2023, 12/05/2023, 19/09/2023, 29/09/2023, 15/12/2023 and 03/06/2024.

Setaria italica, the codon optimised coding sequence for Mpp75Aa1.1 protein from *Brevibacillus laterosporus*, the *HSP-Cl1* 3′ UTR of an *Hsp* gene from *Coix lacryma-jobi*.
  - Between the *DvSnf7.1* suppression cassette and the *mpp75Aa1.1* expression cassette, there is a Isr-1 non-coding intergenic sequence used to minimise potential effects that proximal genes may have on expression.
- The *vpb4Da2* expression cassette consisting of DaMV-2 enhancer from Dalia mosaic virus (DaMV), the *Ltp-Zm1* promoter and leader sequence of a lipid transfer protein gene from *Zea mays*, the *Act-Si1* intron of an actin gene from *Setaria italica*, the codon optimised coding sequence for Vpb4Da2 protein of *Bacillus thuringiensis*, the *SAM1-Si1* 3′ UTR of an *S-adenosylmethionine synthetase 1* gene from *Setaria italica*.
- The *cp4 epsps* expression cassette consisting of the promoter, 5′ UTR and intron sequence of the α-tubulin (TubA) gene from *Oryza sativa*, the chloroplast transit peptide (TS-CTP2) of the *shkG* gene from *Arabidopsis thaliana*, the coding sequence of the *aroA* gene from *Agrobacterium* sp. encoding the CP4 EPSPS protein and the 3′ untranslated sequence of the α-tublin (*TubA*) gene from *Oryza sativa*. The cassette is flanked by *loxP* sites from *Bacteriophage P1*.

The transformation vector PV-ZMOO513642, used to generate the line expressing the Cre recombinase in the HCL617 background, contains two expression cassettes between the right and left border of the T-DNA, containing the following genetic elements:

- the *cre* expression cassette consisting of the promoter, leader, intron and flanking 5′ untranslated sequence of the *act1* gene from *Oryza sativa*, two partial regions of the *cre* recombinase gene from *Bacteriophage P1* interrupted by the second intron sequence from the light inducible (*LS1*) gene from *Solanum tuberosum*, the 3′ untranslated sequence of the *Hsp17* gene from *Triticum aestivum*.
- The *nptII* cassette consisting of the 35S promoter and leader sequence from cauliflower mosaic virus (CaMV), the coding sequence of the *nptII* gene from *Escherichia coli*, the 3′ untranslated sequence of the *nos* gene from *Agrobacterium tumefaciens*.

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 MON 95275 was performed by next-generation sequencing (NGS) and junction sequence analysis (JSA) in order to determine insert copy number and to confirm the absence of the *cp4 epsps* selectable marker, the PV-ZMIR525664 plasmid backbone sequences and the entire PV-ZMOO513642 plasmid and directed sequencing on PCR amplified fragments to determine the size and organisation of the inserted sequences.

The EFSA GMO Panel assessed the sequencing data and found it compliant with the requirements listed in EFSA GMO Panel (2018a), in terms of the approach, of the coverage and sensitivity.

NGS/JSA of the whole genome indicated that maize MON 95275 contains a single insert, consisting of a single copy of the T-DNA in the same configuration as in the PV-ZMIR525664 transformation vector, except for the intended excision of the *cp4 epsps* expression cassette and one *loxP* site. NGS/JSA also indicated the absence of vector backbone sequences.

The nucleotide sequence of the entire insert of maize MON 95275 together with 1000 bp of the 5′ and 1006 bp of the 3′ flanking regions were determined. The insert of 14,682 bp is identical to the T-DNA of PV-ZMIR525664, except for the deletion of 314 bp of the right border region and 200 bp of the left border region. A single nucleotide difference between the MON 95275 and PV-ZMIR525664 sequences was also identified at an intergenic region between the non-coding S-Isr-1 sequence and the DaMV-1 enhancer.

A comparison with the pre-insertion locus indicated that 746 bp were deleted from the maize genomic DNA and 6 bp were co-inserted in the MON 95275 3′ flanking sequence. The possible interruption of known endogenous maize genes by the insertion in maize MON 95275 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 MON 95275. However, the insert is located 1300 bp upstream of the ATG of a putative gene encoding an unknown protein. A northern blot analysis showed that the expression of a DvSnf7.1 dsRNA gives rise to a read-through transcript in leaves that includes part of the uncharacterised endogenous gene, both being in the same orientation. An LC–MS analysis failed to detect a protein corresponding to the putative uncharacterised gene both in the conventional comparator and in the maize MON 95275.

The results of segregation (see Section 3.3.5) and bioinformatics analyses are compatible with a single insertion in the nuclear genome.

Bioinformatics analyses of the amino acid sequence of the newly expressed Mpp75Aa1.1 and Vpb4Da2 proteins reveal no significant similarity to allergens. The bioinformatic analysis to assess similarity to known toxins reveals that Mpp75Aa1.1 displays significant similarity to epsilon-toxin type B (ETX) from *Clostridium perfringens* while Vpb4Da2 displays significant similarity to both protective antigen (PA) from *Bacillus anthracis* and the C2-II component from *Clostridium botulinum*. The relevance of such similarities for food and feed safety is addressed in Section 3.5.

In addition, updated bioinformatic analyses of the newly created open reading frames (ORFs) within the insert and spanning the junctions between the insert and genomic DNA indicate that two short ORFs in frame 2 and 4 presented an eight amino acids perfect match to allergens. However, these ORFs are predicted in different reading frames with respect to the newly expressed proteins and do not contain any known promoter motifs upstream or a start codon. No significant similarities with toxins were identified for any ORF within the insert and spanning the junctions between the insert and genomic DNA. In conclusion, these analyses indicated that the expression of any ORF showing significant similarities to toxins or allergens in maize MON 95275 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 applicant has followed the recommendations by the EFSA GMO Panel for an RNAi off-target search in the plant expressing the DvSnf7 dsRNA, previously assessed in MON 87411 maize (EFSA GMO Panel, 2018b). The DvSnf7.1 dsRNA sequence expressed in maize MON 95275 is identical to the DvSnf7 dsRNA sequence expressed in the single maize event MON 87411, except for the leader sequence. All other RNA sequences in the DvSnf7.1 and DvSnf7 transcripts are identical, including the inverted repeat sequence that forms the active dsRNA. The applicant has followed the recommendations by the GMO Panel for an RNAi off-target search in maize MON 95275 expressing the DvSnf7.1 dsRNA. None of the maize transcript sequences present in the available databases showed perfect match to any of the siRNAs potentially produced. Updated bioinformatics analysis confirms previous results that do not indicate an off-target effect of the DvSnf7 dsRNA expression that would need further assessment. Few maize transcript sequences had regions matching those siRNAs with one to four mismatches. Some of these sequences presented matches for more than one potential siRNA (up to five). 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 DvSnf7.1 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 MON 95275 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 MON 95275 expresses two new proteins: Mpp75Aa1.1 and Vpb4Da2. Given the technical restraints in producing large enough quantities from plants, these proteins were recombinantly produced in *Escherichia coli*. A set of biochemical methods was employed to demonstrate the equivalence between the maize and *E. coli*-derived Mpp75Aa1.1 and Vpb4Da2. Purified proteins from these two sources were characterised and compared in terms of their biochemical, structural and functional properties.

3.3.3.1 | *Mpp75Aa1.1 protein characterisation and equivalence*

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis showed that both plant and microbe-produced Mpp75Aa1.1 proteins had the expected molecular weight of ~35 kDa and were comparably immunoreactive to Mpp75Aa1.1 protein-specific antibodies. Glycosylation analysis demonstrated that none of the Mpp75Aa1.1 proteins were glycosylated. Amino acid sequence analysis of the plant-derived Mpp75Aa1.1 protein by mass spectrometry (MS) methods showed that the protein matched the deduced sequence as defined by the *mpp75Aa1.1* gene. These sequence analysis data were consistent with the previously analysed microbe-produced Mpp75Aa1.1. In addition, the MS data showed that the N-terminal methionine of the plant-produced and *E. coli*-produced Mpp75Aa1.1 protein was truncated. An additional minor population of the bacterial Mpp75Aa1.1 protein with an intact N-terminal methionine was also observed. Such modifications are common in eukaryotic proteins (e.g. Polevoda & Sherman, 2000). Functional equivalence was demonstrated by an in vitro assay which showed that plant and microbe-derived Mpp75Aa1.1 proteins had comparable insecticidal activity.

3.3.3.2 | *Vpb4Da2 protein characterisation and equivalence*

SDS-PAGE and western blot analysis showed that both plant and microbe-produced Vpb4Da2 proteins had the expected molecular weight of ~104 kDa and were comparably immunoreactive to Vpb4Da2 protein-specific antibodies. Glycosylation analysis demonstrated that none of the Vpb4Da2 proteins were glycosylated. Amino acid sequence analysis of the plant-derived Vpb4Da2 protein by MS methods showed that the protein matched the deduced sequence as defined by the *vpb4Da2* gene. These sequence analysis data were consistent with the previously analysed microbe-produced Vpb4Da2. Functional equivalence was demonstrated by an in vitro assay which showed that plant and microbe-derived Vpb4Da2 proteins had comparable insecticidal activity.

The protein characterisation data comparing the biochemical, structural and functional properties of plant and microbe-produced Mpp75Aa1.1 and Vpb4Da2 proteins indicate that these proteins are equivalent, and the microbial derived proteins can be used in the safety studies.

3.3.4 | Information on the expression of the insert

Protein levels of Mpp75Aa1.1 and Vpb4Da2 were analysed by an enzyme-linked immunosorbent assay (ELISA) in material harvested in a field trial across five locations in the Unites States during the 2019 growing season. Analysed samples included grains (R6), forage (R5) and pollen (VT-R1). The mean values, standard errors and ranges of protein expression levels in grains ($n = 20$), forage ($n = 20$) and pollen ($n = 20$) of the Mpp75Aa1.1 and Vpb4Da2 proteins used to estimate human and animal dietary exposure (see Section 3.5.4) are reported in Table 1.

TABLE 1 Mean values, standard errors and ranges of newly expressed proteins in grains, [µg/g dry weight (dw) and µg/g fresh weight (fw)] and forage and pollen [µg/g dry weight (dw)] from maize MON 95275 ($n = 20$).

Tissue	µg/g dry weight (dw)	µg/g fresh weight (fw)
Grains (R6)		
Mpp75Aa1.1	1.3[a] ± 0.086[b] (0.67–1.9)[c]	1.1 ± 0.076 (0.59–1.7)
Vpb4Da2	1.2 ± 0.086 (0.42–1.9)	1.0 ± 0.076 (0.37–1.6)
Forage (R5)		
Mpp75Aa1.1	16 ± 0.76 (12–25)	
Vpb4Da2	3.3 ± 0.13 (2.5–4.8)	
Pollen (VT-R1)		
Mpp75Aa1.1	< LOQ[d]	
Vpb4Da2	< LOQ[d]	

[a]Mean value.
[b]Standard deviation.
[c]Range.
[d]All samples were below the limit of quantification (LOQ for Mpp75Aa1.1 in pollen = 0.125 µg/g dw; LOQ for Vpb4Da2 in pollen = 0.157 µg/g dw).

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 DvSnf7.1 dsRNA were not considered relevant for the risk assessment of maize MON 95275.

3.3.5 | Inheritance and stability of inserted DNA

Genetic stability of maize MON 95275 insert was assessed by NGS/JSA from five generations (F4, F4F1, F5, F5F1, F6) and PCR-based segregation analysis from three generations (F4F2, F4F3, F4F4). The results indicate that all the plants tested retained a single copy of the insert and flanking regions, which were stably inherited in subsequent generations. The results support the presence of a single insertion, segregating in a Mendelian fashion.

3.3.6 | Conclusion on molecular characterisation

The molecular characterisation data establish that maize MON 95275 contains a single insert consisting of one copy of the *mpp75Aa1.1*, the *vpb4Da2* and the *DvSnf7.1* expression cassettes. Bioinformatic analyses of the sequences encoding the two newly expressed proteins and other ORFs within the insert or spanning the junctions between the insert and genomic DNA reveal similarity to known toxins, which required further food/feed safety assessment. The in planta RNAi off-target search, performed with the sequence of the DvSnf7.1 dsRNA, does 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 Mpp75Aa1.1 and Vpb4Da2 proteins is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of plant and microbe-produced Mpp75Aa1.1 and Vpb4Da2 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 GMFF-2022-5890 presents data on agronomic and phenotypic characteristics, as well as on forage and grain composition of maize MON 95275 (Table 2). In addition, the application contains data on pollen characteristics and morphology from maize MON 95275 (Appendix A).

TABLE 2 Main comparative analysis studies to characterise maize MON 95275 provided in application GMFF-2022-5890.

Study focus	Study details	Comparator	Non-GM reference varieties
Agronomic and phenotypic compositional analysis	Field study, US, 2019, eight sites[a]	LH244 × HCL617	17[b]

Abbreviation: GM, genetically modified.

[a]Three field trials were located in Illinois, two field trials were located in Iowa and one field trial was located in each of the following states: Indiana, Missouri and Nebraska.

[b]The following maize hybrids were used (comparative relative maturity in brackets): Brodbeck 1806 (106), Dekalb DKC61-52 (111), Dekalb DKC64-85 (114), Dekalb DKC65-18 (115), Fontanelle 10C616 (110), Golden Harvest G07F23 (107), Golden Harves G10T63 (110), Hubner H3844 (115), Mycogen 2H721 (112), Mycogen 2J790 (114), Pioneer P0993 (109), Pioneer P1345 (113), Stewart S480 (106), Stewart S660 (113), Stewart S750 (114), Stine 9706–0 (111) and Stone 5420 (113).

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 MON 95275, the comparator LH244 × HCL617 and four non-GM reference varieties.

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

3.4.3 | Suitability of selected test materials

3.4.3.1 | *Selection of the test materials*

As described in Section 3.3.1, inbred line LH244 was transformed to obtain line MON 95275, which was then crossed with the inbred line HCL617 to produce the hybrid maize MON 95275[12] used in the comparative analysis.

The comparator used in the field trials is the non-GM maize hybrid LH244 × HCL617, which is isogenic to hybrid maize MON 95275 (as documented by the pedigree) and is considered to be the conventional counterpart.

Both hybrid maize MON 95275 and the conventional counterpart have a comparative relative maturity (CRM) of 111, which is considered appropriate for growing in environments across the US, where the comparative field trials were conducted.

Commercial non-GM reference varieties with a CRM ranging from 106 to 115 were selected by the applicant and, at each selected site, four reference varieties were tested (see Table 2). 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*

Seeds of maize MON 95275 and the conventional counterpart used in the 2019 field trials were produced, harvested and stored under similar conditions, before being sown in the field trial sites. The seed lots were verified for their identity via event-specific quantitative PCR analysis.

The grains were tested for their germination capacity under optimal and suboptimal temperature conditions.[13] The germination capacity of the GM maize MON 95275 was compared with the one of its conventional counterpart and four reference maize hybrids.[14] The results of these studies indicate that the seed germination of maize MON 95275 was not different than its conventional counterpart.[15]

[10]Dossier: Part II – Section 1.3; additional information: 17/2/2023 and 26/7/2023.

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

[12]For the agronomic, phenotypic and compositional analysis, hybrid maize MON 95275 refers to the event obtained crossing inbred line MON 95274 in LH244 with the inbred line HCL617.

[13]Optimal temperature condition corresponds to approximately 25°C and suboptimal 10°C for 7 days followed by 4 days at 25°C.

[14]These were: Hubner H3844; Stine9815-0; Dekalb DKC64-85; Golden Harvest G07F23.

[15]GM hybrid maize showed a mean germination of 99.3% under both temperature conditions while its conventional counterpart showed a mean of 98.3% and 99% under optimal and suboptimal temperature conditions, respectively.

3.4.3.3 | *Conclusion on suitability*

The GMO Panel is of the opinion that maize MON 95275, the conventional counterpart and the non-GM maize reference hybrids were properly selected and are of adequate quality. Therefore, the test materials are considered suitable for comparative analysis.

3.4.4 | Representativeness of the receiving environments

3.4.4.1 | *Selection of field trial sites*

The selected field trials sites were located in commercial maize-growing regions of the United States. The soil and climatic characteristics of the selected fields[16] correspond to optimal, near-optimal and suboptimal conditions for maize cultivation (Sys et al., 1993).

The GMO Panel considers that the selected sites reflect commercial maize-growing regions in which the test materials are likely to be grown.

3.4.4.2 | *Meteorological conditions*

Maximum and minimum mean temperatures and sums of precipitation were provided for each site on a weekly basis. No exceptional weather conditions were reported at any of the selected sites; therefore, the GMO Panel considers that the meteorological data set falls within the historical range of climatic conditions normally occurring at these sites.

3.4.4.3 | *Management practices*

The field trials included plots containing maize MON 95275, plots with the conventional counterpart and plots with non-GM maize reference varieties, mostly managed according to local agricultural practices.

Despite not being considered a normal agricultural practice, thinning was applied at one field trial site located in Iowa to achieve a more homogeneous plant density across plots. The GMO Panel considers that the management practices including sowing, harvesting and application of plant protection product were acceptable for the selected receiving environments.

3.4.4.4 | *Conclusion on representativeness*

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

3.4.5 | Agronomic and phenotypic analysis

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

The statistical analysis (Section 3.4.2) was applied to 10 endpoints, with the following results:

- For maize MON 95275, the test of difference identified statistically significant differences with the conventional counterpart for early stand count, days to flowering, plant height and ear height. All these endpoints fell under equivalence category I.

[16]Soil types of the field trials were clay loam, silty clay loam, silt loam, loam and sandy loam; soil organic matter ranged from 0.6% to 3.0%; pH ranged from 6.2 to 7.4; average temperatures and sum of precipitations during the usual crop growing season ranged respectively from 15.9°C to 24.8°C and from 257 to 892 mm.

[17]Early stand count, days to flowering, plant height, ear height, days to maturity, lodging, fruit count, final stand count, moisture, yield and seed weight.

3.4.6 | Compositional analysis

Maize MON 95275 forage and grain harvested from eight sites (Table 2) were analysed for 78 constituents (nine in forage and 69 in grains), including those recommended by OECD (OECD, 2002). The statistical analysis was not applied to 15 grain constituents because their concentration in more than half of the samples were below the limit of quantification.[18]

The statistical analysis was applied to a total of 63 constituents (nine in forage[19] and 54 in grain[20]); a summary of the outcome of the test of difference and the test of equivalence is presented in Table 3.

For MON 95275 maize, statistically significant differences in the comparison with its conventional counterpart were found for 11 endpoints (one in forage and 10 in grains). All these endpoints fell under equivalence category I or II.

TABLE 3 Outcome of the comparative compositional analysis in forage and grains from maize MON 95275. The table shows the number of endpoints in each category.

		Test of difference[a]	
		Not different	**Significantly different**
Test of equivalence[b]	Category I/II	52	11[c]
	Category III/IV	–	–
	Not categorised	–	–
	Total endpoints	63	

[a]Comparison between maize MON 95275 and its conventional counterpart.

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

[c]Endpoints with significant differences between maize MON 95275 and its conventional counterpart falling in equivalence category I-II. For forage: protein. For grains: palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), arachidic acid (C20:0), behenic acid (C22:0), calcium, potassium, thiamine, pyridoxine.

The GMO Panel assessed all the significant differences between the maize MON 95275 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. No endpoints were identified that showed significant differences between the MON 95275 maize and its conventional counterpart and fell under category III/IV.

3.4.7 | Conclusion on comparative analysis

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

Considering 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 MON 95275 and its conventional counterpart needs further assessment regarding their potential environmental impact.
- None of the differences identified in forage and grain composition between the maize MON 95275 and its conventional counterpart needs further assessment regarding food and feed safety.

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

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

[20]Proximates and fibre content (ash, carbohydrates, total fat, protein, moisture, acid detergent fibre (ADF), neutral detergent fibre (NDF) and total dietary fibre (TDF)), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, zinc), vitamins (β-carotene, α-tocopherol, thiamine, riboflavin, niacin, pyridoxine, folic acid), amino acids (alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine), fatty acids (palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), α-linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C20:1), behenic acid (C22:0)) and other compounds (p-coumaric acid, ferulic acid, phytic acid, raffinose).

3.5 | Food/feed safety assessment[21]

3.5.1 | Overview of overarching information for food/feed assessment

3.5.1.1 | *Compositional analysis*

The compositional analysis of maize MON 95275 and its conventional counterpart provided by the applicant and assessed by the GMO Panel is described in Section 3.4.6.

3.5.1.2 | *Newly expressed proteins and other new constituents (RNAi)*

Two insecticidal proteins, Mpp75Aa1.1 and Vpb4Da2, are newly expressed in maize MON 95275 and have never been previously assessed by the GMO Panel (for details, see below). In addition, the DvSnf7.1 dsRNA and derived siRNAs are also newly expressed in maize MON 95275 but have been previously assessed by the GMO Panel in previous applications (for details see Section 3.5.2.2).

3.5.1.2.1 | *Molecular characterisation of the NEPs*

The Mpp75Aa1.1 and Vpb4Da2 proteins in maize MON 95275 have been extensively characterised. Furthermore, the equivalence between the maize and *E. coli*- derived Mpp75Aa1.1 and Vpb4Da2 proteins was demonstrated (see Section 3.3.3).

3.5.1.2.2 | *History of safe use for consumption as food/feed of the NEPs*

a. Information on the source organism

The *Mpp75Aa1* gene was isolated from the microorganism *Brevibacillus laterosporus* that has been found in several foods (e.g. Iurlina & Fritz, 2005; Panda et al., 2014; Sarkar et al., 2002). *B. laterosporus* is also used as a commercial probiotic, human dietary supplement and feed additive (Liu et al., 2023; Liu et al., 2024; Wang et al., 2022). However, the amount of Mpp75Aa1.1 protein consumption by humans and animals through exposure to *B. laterosporus* has not been established.

Vpb4D2 is a naturally occurring insecticidal protein from *B. thuringiensis*, which is widely applied as a microbial biopesticide to many agricultural commodities implying a solid history of exposure. However, the amount of Vpb4Da2 protein being consumed by humans and animals has not been established.

b. Information on structure, function and mode of action

The full-length Mpp75Aa1 protein displays a 23-amino acid N-terminal membrane-transiting signal peptide. Mpp75Aa1.1 constitutes the mature form of the protein without these N-terminal 23 amino acids. It is a member of the ETX_MTX2 family of membrane pore-forming toxins that are composed of three structural parts: a receptor binding domain, an oligomerisation domain and a membrane pore-forming domain. Mpp75Aa1.1 shares a common mode of action with the traditional insecticidal Cry proteins derived from *B. thuringiensis*. After proteolytic processing at its C terminus by midgut proteases, the activated Mpp75Aa1.1 protein forms an oligomer that specifically interacts with a not yet identified membrane-associated receptor on the midgut microvilli to cause cellular damage and target insect death (Kouadio, Duff, et al., 2021). Mpp75Aa1.1 displays structural similarity to the pore-forming epsilon toxin (another member of the ETX_MTX2 family) that is produced by certain strains of *Clostridium perfringens* and causes enterotoxaemia mainly in goats and sheep. However, the receptor-binding domain of epsilon toxin is different from the putative receptor-binding domain of Mpp75Aa1.1, which suggests that these pore-forming proteins bind to distinct membrane receptors, thus potentially explaining the expected specificity of Mpp75Aa1.1 for target insects (Kouadio, Duff, et al., 2021).

The Vpb4Da2 protein is a member of the Bacterial_exotoxin_B family of membrane pore-forming toxins and is composed of six structural domains. It shares a common mode of action with the traditional insecticidal Cry proteins derived from *B. thuringiensis*. After proteolytic processing by midgut proteases, the activated Vpb4Da2 protein forms an oligomer that specifically interacts with a not yet identified membrane-associated receptor on the midgut microvilli to cause cellular damage and target insect death (Kouadio, Zheng, et al., 2021). The N-terminal part of Vpb4Da2 (domains 1–3) displays structural similarity to the *Bacillus anthracis* protective antigen (PA) protein and the *Clostridium botulinum* C2-II component, which are involved in the pathogenesis of the respective bacterial toxins in humans and animals by translocating toxic enzymes into cells. However, the C-terminal part of Vpb4Da2 protein (domains 4–6), comprising the putative membrane receptor-binding domain, is structurally different from the receptor-binding domains of *B. anthracis* PA and *C. botulinum* C2-II proteins, suggesting that these factors recognise distinct membrane receptors, which potentially explains the expected specificity of Vpb4Da2 for target insects (Kouadio, Zheng, et al., 2021). Furthermore, PA and C2-II proteins are both

[21]Dossier: Part II – Sections 1.4, 1.5, 1.6, 2, 3 and 4; additional information: 07/11/2022, 17/02/2023, 26/07/2023, 29/09/2023, 15/12/2023, 12/01/2024, 28/03/2024 and 10/06/2024.

non-toxic subunits that, unlike Vpb4Da2 protein, require a specific binary partner to exert their pore forming activity (see Section 3.5.2.1.2).

c. Information on identity/homology of NEPs to other proteins/constituents in conventional food and feed sources

No information on identity/homology of Mpp75Aa1.1 and Vpb4Da2 proteins to other proteins in conventional food and feed sources was provided by the applicant.

d. Overall conclusion of the history of safe use

Although there is familiarity with the Mpp75Aa1.1 and Vpb4Da2 proteins, these two insecticidal proteins share structural similarities with bacterial toxins or toxin components that result in diseases in humans and animals. Therefore, the GMO Panel considers that the available information on the history of use is not sufficient to conclude on the food and feed safety of the Mpp75Aa1.1 and Vpb4Da2 proteins.

3.5.1.2.3 | *Stability of the NEPs*

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

a. Effect of temperature and pH on NEPs

The applicant provided experimental studies on the effects of temperature on the Mpp75Aa1.1 and Vpb4Da2 proteins as expressed in maize MON 95275, using a microbial recombinant system. Independent samples of the Mpp75Aa1.1 and Vpb4Da2 proteins were incubated for 15 or 30 min at 25°C, 37°C, 55°C, 75°C and 95°C followed by SDS-PAGE or by a bioassay measuring their functional activity. The studies showed that the functional activity of the Mpp75Aa1.1 and Vpb4Da2 proteins was visibly diminished at temperatures of 95°C and 75°C, respectively.

In relation to the effect of pH on the Mpp75Aa1.1 and Vpb4Da2 proteins, the molecular mass and immunoreactivity of the proteins were unchanged at pH 1.2 and 7.5.

b. In vitro protein degradation by proteolytic enzymes

The applicant provided independent studies on in vitro protein degradation (i.e. resistance to pepsin in solutions at pH ~ 1.2) of the Mpp75Aa1.1 and Vpb4Da2 proteins as expressed in maize MON 95275. The integrity of the test Mpp75Aa1.1 and Vpb4Da2 proteins in samples incubated at various time points was analysed by SDS-PAGE followed by protein staining or by Western blotting. Samples of the Mpp75Aa1.1 and Vpb4Da2 proteins were degraded by pepsin within less than 0.5 min of incubation. In these two studies, peptide fragments from ~ 2.5 to 6 kDa were visible after 0.5 and 2 min of incubation but disappeared at the 5 min time point.

Furthermore, the applicant provided an analysis of the resistance to degradation by pancreatin in solutions at pH ~ 7.5. The Mpp75Aa1.1 and Vpb4Da2 proteins were degraded after 15 min and 5 min of incubation, respectively, when analysed by western blotting.

3.5.1.2.4 | *Synergistic or antagonistic interactions*

Two proteins (Mpp75Aa1.1 and Vpb4Da2) are newly expressed in maize MON 95275. The potential for a functional interaction among them has been assessed with regard to human and animal health. These insecticidal proteins act through cellular receptors in target insect species. It is reported that the gastrointestinal tract of mammals, including humans, lacks receptors with high specific affinity to these proteins (Kouadio, Duff, et al., 2021; Kouadio, Zheng, et al., 2021). 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 MON 95275 (Table 4).

TABLE 4 Intended effects of the NEPs in maize MON 95275.

Protein	Intended effect in GM plant
Mpp75Aa1.1	The Mpp75Aa1.1 protein provides protection against western corn rootworm and shares a similar mode of action to Cry proteins (Kouadio, Duff, et al., 2021)
Vpb4Da2	The Vpb4Da2 protein is a *B. thuringiensis* vegetative insecticidal protein, active against western corn rootworm, via a mechanism similar to Cry proteins (Kouadio, Zheng, et al., 2021)

3.5.1.3 | *Effect of processing*

Maize MON 95275 will undergo existing production processes used for conventional maize. No novel production process is envisaged. Based on the outcome of the comparative assessment, processing of the GM maize into food and feed products is not expected to result in products being different from those of conventional non-GM maize varieties.

3.5.2 | Toxicology assessment

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

3.5.2.1 | *Assessment of newly expressed proteins*

A weight-of-evidence approach was followed by the GMO Panel to assess the toxicological profile of the newly expressed Mpp75Aa1.1 and Vpb4Da2 proteins, taking into account all of the information relevant for their hazard assessment, including molecular characterisation, substrate specificity, history of safe use for consumption as food and feed of the NEPs, stability of the NEPs and synergistic or antagonistic interactions (Section 3.5.1.2), updated bioinformatic analyses for similarity to toxins and in vivo toxicity studies.

3.5.2.1.1 | *Bioinformatics*

Updated bioinformatic analyses of the amino acid sequences of Mpp75Aa1.1 and Vpb4Da2 proteins have revealed homology to known toxins. Mpp75Aa1.1 protein exhibits similarity to Q02307, epsilon-toxin type B (ETX) from *Clostridium perfringens*, displaying 25.9% identity over 304 amino acids. Vpb4Da2 protein shows homology to P13423, protective antigen (PA) from *Bacillus anthracis*, with 32.7% identity over 626 amino acids. In addition, the homology between Vpb4Da2 and *C. botulinum* C2-II proteins (entry BAA32537.1 in the NCBI database), displaying 35.5% identity over 681 amino acids, was also assessed.

The Mpp75Aa1.1 and Vpb4Da2 proteins provide protection against western corn rootworm, sharing a similar mode of action to Cry proteins which is reported to have a high specificity for insect and nematode gut (Kouadio, Duff, et al., 2021; Kouadio, Zheng, et al., 2021).

In relation to the Mpp75Aa1.1 protein and the homology to Q02307 (ETX), the sequences associated with receptor binding and toxin specificity are localised in Domain I and cannot be overlapped with those of Mpp75Aa1.1 due to a low structural similarity. Inspection of the amino acid sequence alignment showed that the receptor binding regions from Domain I of Q02307 and Mpp75Aa1.1 share the least homology (19% sequence identity) of all regions of this alignment. Several key amino acids including Y29, Y30, Y36, H149, Y196 and F199 in Q02307 have been identified as critical for receptor binding and mammalian toxicity (Moar et al., 2017), but only two out of these six amino acids are present in Mpp75Aa1.1. In addition, the head-to-head comparison between the crystal structures of Mpp75Aa1.1 and ETX reveals significant differences in the receptor binding domain and the C-terminal end of the proteins (Kouadio, Duff, et al., 2021).

The Vpb4Da2 domains that confer host-specificity (i.e. domains 4–6) exhibit a low degree of sequence conservation within the same protein family (Kouadio, Zheng, et al., 2021). The low degree of sequence conservation in Vpb4Da2 domains 4–6 suggests these domains are involved in providing specific host recognition. Thus, the amino acid sequence alignment of the receptor binding domains of Vpb4Da2 and PA or C2-II proteins revealed that a significant portion of them were unaligned. Likewise, the comparison of Vpb4Da2 three-dimensional structure with that of PA revealed that domains 5 and 6 are unique to Vpb4Da2 protein whereas Vpb4Da2 domain 4 displayed a distinct topology (Kouadio, Zheng, et al., 2021). Furthermore, PA and C2-II proteins are the binding/translocation components of respective binary proteins requiring the presence of other enzymatic components, i.e. lethal (LF) or oedema factors (EF) for PA and C2-I subunit in the case of C2-II, to cause cellular toxicity (Schleberger et al., 2006; Sherer et al., 2007). In agreement with this, several studies have shown that both PA and C2-II proteins are non-toxic in the absence of their respective binary partners (Collier & Young, 2003; Friedlander, 1986; Heber et al., 2023). Moreover, both PA and C2-II proteins possess a conserved phenylalanine residue at position 425, which is a critical functional amino acid of the φ-clamp to catalyse the translocation of their corresponding enzymatic components (i.e. LF and EF or C2-I proteins) across the membrane (Neumeyer et al., 2008; Sun et al., 2008). The amino acid sequence alignment showed that Vpb4Da2 has an alanine residue at position 425, suggesting that it is unlikely to translocate other proteins into host cells following the same mechanism as observed for the binary toxins (Kouadio, Zheng, et al., 2021).

3.5.2.1.2 | *in vitro studies*

The Mpp75Aa1.1 and Vpb4Da2 proteins are degraded by pepsin and pancreatin (Section 3.5.1.2.3).

3.5.2.1.3 | *In vivo toxicity studies*

For the assessment of the Mpp75Aa1.1 and Vpb4Da2 proteins, the applicant provided a 28-day toxicity study with each protein, two acute toxicity studies with Mpp75Aa1.1 protein and one acute toxicity study with Vpb4Da2 protein. The outcome of the in vivo toxicity studies with the Mpp75Aa1.1 and Vpb4Da2 proteins is described below.

Acute toxicity studies

In two acute toxicity studies with similar methodology, CD-1 mice were administered the *E. Coli*-produced Mpp75Aa1.1[22] protein by gavage at the dose of 2000 mg/kg (bw) showed no adverse effects.

An acute toxicity study in CD-1 mice administered the *E. Coli*-produced Vpb4Da2 protein by gavage at the dose of 5000 mg/kg (bw) showed no adverse effects.

28-day repeated dose toxicity study with Mpp75Aa1.1 protein

The 28-day toxicity study in mice with the Mpp75Aa1.1 protein was conducted in accordance with OECD TG 407 (2008) and to the principles of good laboratory practice.

Crl:CD-1 mice (20/sex per group), 8- to 9-week-old at the start of dosing were allocated to five groups. Groups were administered by oral gavage: the test substance (Mpp75Aa1.1 protein) at targeted nominal doses of 600, 60 or 6 mg/kg body weight (bw) per day (high, medium and low Mpp75Aa1.1 protein groups); 600 mg/kg bw per day of bovine serum albumin (BSA control group); and the vehicle.

Efforts to modify the dosing solution to reduce the viscosity at high concentrations of Mpp75Aa1.1, which would permit dosing at up to 1000 mg/kg bw per day, were unsuccessful. The high viscosity was investigated and considered to be related to the physical chemical properties of Mpp75Aa1.1. The GMO panel concludes that in the case of Mpp75Aa1.1, the dose of 600 mg/kg bw per day is the highest that could realistically be achieved by gavage and therefore this 28-day study is acceptable.

Mice were randomised to treatment groups (males and females separately) using a stratified randomisation block designed to achieve similar group mean body weights (± 20% of the mean for each sex). Due to behavioural characteristics, animals were singly housed. The GMO Panel considers this justification acceptable. The test substance used in this study was produced by a recombinant system and contained about 100% Mpp75Aa1.1 protein. The amino acid sequence analysis of the *E. coli*-produced Mpp75Aa1.1 used in this 28-day toxicity study by mass fingerprint analysis matched the deduced sequence as defined by the *mpp75Aa1.1* gene. This protein had the expected molecular weight and immunoreactivity to Mpp75Aa1.1 specific antibodies, was not glycosylated and showed functional activity. The first 10 animals per group were subject to in-life procedures and observations and terminal procedures in accordance to OECD TG 407 (2008), except coagulation analysis; the remaining 10 animals per group were used to evaluate coagulation parameters, body weight, food consumption and clinical observation parameters only. Deviations to the protocol reported in the study were considered minor deviations with no impact on the study results.

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

There were no Mpp75Aa1.1-related incidents of mortality or clinical signs. No Mpp75Aa1.1 -related adverse findings were identified in any of the investigated parameters. No Mpp75Aa1.1-related clinical observations or ophthalmology findings were seen.

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 endpoints;
- exhibited no consistency with increasing dose levels.

No gross pathology findings related to the administration of the test item 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 item compared to the controls.

[22]As clarified in Part II Sci. info. 1.2 Molecular Characterisation, the Cry75Aa1.1 referred to in the acute study has been recently reclassified as Mpp75Aa1.1 to distinguish α-PFPs (e.g. Cry 1, 2 and 3) from β-PFPs (e.g. Mpp).

[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 treatment-related account is taken of whether the result in the test group is outside the normal range for untreated animals of the same strain and age. To do this, a number of sources of information are considered, including the standardised effect size, the standard deviations and range of values within test and control groups in the study and, if applicable, data from other studies performed in the same test facility within a small timeframe and under almost identical conditions (Historic Control Data).

The GMO panel concludes that no adverse effects were observed in this 28-day mouse toxicity study on the microbially produced Mpp75Aa1.1 protein, at doses up to 600 mg/kg bw per day.

28-day toxicity study with Vpb4Da2 protein

The 28-day toxicity study in mice with the Vpb4Da2 protein was conducted in accordance with OECD TG 407 (2008) and to the principles of good laboratory practice.

Crl:CD-1 mice (20/sex per group), 8- to 9-week-old at the start of dosing were allocated to five groups. Groups were administered by oral gavage: the test substance (Vpb4Da2 protein) at targeted nominal doses of 1000, 100 or 10 mg/kg body weight (bw) per day (high, medium and low Vpb4Da2 protein groups); 1000 mg/kg bw per day of bovine serum albumin (BSA control group); and the vehicle.

Mice were randomised to treatment groups (males and females separately) using a stratified randomisation block designed to achieve similar group mean body weights (± 20% of the mean for each sex).

Due to behavioural characteristics, animals were singly housed. The GMO Panel considers this justification acceptable.

The test substance used in this study was produced by a recombinant system and contained about 97% Vpb4Da2 protein. The amino acid sequence analysis of the *E. coli*-produced Vpb4Da2 used in this 28-day toxicity study by mass fingerprint analysis matched the deduced sequence as defined by the *vpb4Da2* gene. This protein had the expected molecular weight and immunoreactivity to Vpb4Da2 specific antibodies, was not glycosylated and showed functional activity.

The first 10 animals per group were subject to in-life procedures and observations and terminal procedures in accordance with OECD TG 407 (2008), except coagulation analysis; the remaining 10 animals per group were used to evaluate coagulation parameters, body weight, food consumption and clinical observation parameters only.

Deviations to the protocol reported in the study were considered minor deviations with no impact on the study results.

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

There were no Vpb4Da2-related incidents of mortality or clinical signs. No Vpb4Da2-related adverse findings were identified in any of the investigated parameters. No Vpb4Da2-related clinical observations or ophthalmology findings were seen.

A small number of statistically significant findings were noted but these were not considered adverse effects of treatment for one or more of the following reasons:

- were within the normal variation[24] for the parameter in mice of this age;
- were of small magnitude;
- were identified at only a small number of time intervals with no impact on the overall value;
- exhibited no consistent pattern with related parameters or endpoints;
- exhibited no consistency with increasing dose levels.

No gross pathology findings related to the administration of the test item 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 item compared to the controls.

The GMO Panel concludes that no adverse effects were observed in this 28-day mouse toxicity study on the microbially produced Vpb4Da2 protein, at doses up to 1000 mg/kg bw per day.

3.5.2.1.4 | *Overall conclusion of toxicological assessment of the NEPs*

Based on the above information, the GMO Panel did not identify indications that the Mpp75Aa1.1 and Vpb4Da2 proteins raise food and feed safety concerns in humans and animals.

Taking the protein degradation data, the absence of adverse effects in a 28-day toxicity study, evidence for high specificity to the target organism and requirement for the presence of additional proteins for toxicity together, the GMO panel concludes that the weight of evidence is that Vpb4Da2 and Mpp75Aa1.1 proteins are unlikely to act like anthrax, *C. botulinum* and *C. perfringens* toxins. Therefore, the hits of Vpb4Da2 and Mpp75Aa1.1 proteins with the *Bacillus anthracis* 'Protective antigen (PA)', *C. botulinum* C2-II and *C. perfringens* ETX proteins, respectively, resulting from bioinformatics analysis are considered to be of no safety concern.

[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 treatment-related account is taken of whether the result in the test group is outside the normal range for untreated animals of the same strain and age. To do this, a number of sources of information are considered, including the standardised effect size, the standard deviations and range of values within test and control groups in the study and, if applicable, data from other studies performed in the same test facility within a small timeframe and under almost identical conditions (Historic Control Data).

3.5.2.2 | *Assessment of new constituents other than NEPs*

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 forage and grain from maize MON 95275, with the exception of the intended expression of DvSnf7.1 dsRNA and derived siRNAs, designed to control coleopteran pests via RNAi. According to the applicant, the DvSnf7.1 dsRNA sequence expressed in maize MON 95275 is identical to the DvSnf7 dsRNA sequence expressed in the single maize event MON 87411, except from the leader sequence. All other RNA sequences in the DvSnf7.1 and DvSnf7 transcripts are identical, including the inverted repeat sequence that forms the active dsRNA.

The GMO Panel has previously assessed DvSnf7 dsRNA in the context of the single maize event MON 87411 (EFSA GMO Panel, 2018b) and concluded that no safety concerns are associated with their presence. The GMO Panel is not aware of any new information that would change its previous conclusions on the safety of these compounds. On the basis of the known biological function of these constituents, there is currently no expectation for possible interactions with other new compounds (newly expressed proteins) or other constituents relevant to the food and feed safety of maize MON 95275.

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

Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, none of the differences identified between maize MON 95275 and its conventional counterpart in grains and forage composition require further assessment.

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

Based on the outcome of the molecular characterisation, comparative analysis and toxicological assessment, no indications of findings relevant to food and feed safety have been identified for maize MON 95275 related to the stability and expression of the insert and to modifications of toxicological concern in the composition of maize MON 95275 (see Sections 3.3, 3.4 and 3.5). Therefore, animal studies with food/feed derived from maize MON 95275 are not considered necessary by the GMO Panel (EFSA GMO Panel, 2011a). In accordance with Regulation (EU) No 503/2013, the applicant provided a 90-day feeding study in rats fed with diets containing grains derived from maize MON 95275.

In this study, pair-housed Crl:CD (SD) rats (16 per sex per group; 2 rats per cage) were allocated to 3 groups using a randomised complete block design with 8 replications per sex.

Groups were fed diets containing maize MON 95275 grains at 50% and 33% of inclusion level (the latter supplemented with 17% of the conventional counterpart maize) and the conventional counterpart (inclusion level 50%).

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

The stability of the test and control materials was not verified; however, in accordance to 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.

Event-specific PCR analysis confirmed the presence of the event MON 95275 in both the GM grains and diets and excluded the presence of the event in the respective controls.

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

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

An appropriate range of statistical tests were performed on the results of the study. Detailed description of the methodology and of statistically significant findings identified in rats given diets containing grains derived from maize MON 95275 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[25] for the parameter in rats of this age;
- were of small magnitude;

[25]Although animal 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 treatment-related account is taken of whether the result in the test group is outside the normal range for untreated animals of the same strain and age. To do this, a number of sources of information are considered, including the standardised effect size, the standard deviations and range of values within test and control groups in the study and, if applicable, data from other studies performed in the same test facility within a small timeframe and under almost identical conditions (Historic Control Data).

- were identified at only a small number of time intervals with no impact on the overall value;
- exhibited no consistent pattern with related parameters or endpoints.
- exhibited no consistency with increasing incorporation levels.

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

The GMO Panel concludes that this study is in line with the requirements of Regulation (EU) No 503/2013 and that no treatment-related adverse effects were observed in rats after feeding diets containing maize MON 95275 grains at 33% or 50% for 90 days.

3.5.3 | Allergenicity

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

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

A weight-of-evidence approach was followed, taking into account all 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, 2017; Regulation (EU)No 503/2013).

The *mpp75Aa1.1* and *vpb4Da2* genes originate from *B. laterosporus* and *B. thuringiensis,* respectively, none of which are considered common allergenic sources.

Updated bioinformatic analyses of the amino acid sequences of the Mpp75Aa1.1 and Vpb4Da2 proteins, using the criterion of more than 35% identity in a sliding window of 80 amino acids, revealed no relevant similarities to known allergens.

The studies on protein stability of the Mpp75Aa1.1 and Vpb4Da2 proteins have been described in Section 3.5.1.2.3. In addition, the GMO Panel did not find an indication that the newly expressed proteins Mpp75Aa1.1 and Vpb4Da2 at the levels expressed in maize MON 95275 might be adjuvants.

Furthermore, the applicant provided information on the safety of the Mpp75Aa1.1 and Vpb4Da2 proteins regarding their potential hazard to cause a celiac disease response.[26] For such assessment, the applicant followed the principles described in the EFSA GMO Panel guidance document (EFSA GMO Panel, 2017). The assessment of the Mpp75Aa1.1 and Vpb4Da2 proteins identified no perfect or relevant partial matches with known celiac disease peptide sequences. Therefore, no indications of safety concerns were identified by the GMO Panel.

In the context of this application, the GMO Panel considers that there are no indications that the newly expressed Mpp75Aa1.1 and/or Vpb4Da2 proteins in GM maize MON 95275 may be allergenic.

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

The GMO Panel regularly reviews the available publications on food allergy to maize. However, maize is not considered a common allergenic food[27] (OECD, 2002). Therefore, the GMO Panel does not request experimental data as a routine basis 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 a potentially increased allergenicity of food and feed derived from this GM maize MON 95275 with respect to that derived from the conventional counterpart and the non-GM reference varieties tested.

3.5.4 | Dietary exposure assessment to new constituents

In line with Regulation (EU) No 503/2013, the applicant provided dietary exposure estimates to Mpp75Aa1.1 and Vpb4Da2 proteins newly expressed in maize MON 95275. Dietary exposure was estimated based on protein expression levels

[26]Technical dossier Section 1.5, additional information 30/06/2022.

[27]Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

reported in this application for maize MON 95275, 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 MON 95275 grains, forage and pollen were derived from replicated field trials (four replicates from five locations) in 2019 in the United States (Table 1, Section 3.3.4).

Human and animal dietary exposure assessment to DvSnf7 dsRNA and its derived siRNAs was not conducted because these molecules are generally rapidly denaturated, depurinated and degraded shortly after ingestion, and therefore, they are considered generally not to exert any biological effects once ingested by humans and animals (EFSA GMO Panel, 2018b).

3.5.4.1 | *Human dietary exposure*

Chronic and acute dietary exposure to Mpp75Aa1.1 and Vpb4Da2 proteins newly expressed in MON 95275 maize were provided. The applicant followed the methodology described in the EFSA Statement 'Human dietary exposure assessment to newly expressed protein in GM foods' to anticipate human dietary exposure making use of summary statistics of consumption (EFSA, 2019a). Human dietary exposure was estimated across 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 in MON 95275 maize grains, a conservative scenario with 100% replacement of conventional maize by the GM maize was considered. Consumption figures for all relevant commodities (e.g. corn flakes, sweet corn, popcorn, etc.) were retrieved from the EFSA Comprehensive European Food Consumption Database (EFSA consumption database).[28] Corn oil, corn starch and corn syrup were excluded from the assessment since no proteins are expected to be present in these commodities.

Mean protein expression values on fresh weight basis are considered as the most adequate to estimate human dietary exposure (both acute and chronic) when working with raw primary commodities that are commonly consumed as processed blended commodities (EFSA, 2019a). Different recipes and factors were considered to estimate the amount of maize in the consumed commodities before assigning newly expressed protein levels to the relevant commodities.[29] No losses in the newly expressed proteins during processing were considered, except for the commodities mentioned above.

The highest anticipated acute dietary exposure (highly exposed population) was estimated in the age class 'Other children' with estimates of 16.7 μg/kg bw per day and 15.2 μg/kg bw per day for Mpp75Aa1.1 and Vpb4Da2 proteins, respectively. The main contributor to the exposure in the dietary survey with the highest estimates would be corn grains.

The highest anticipated chronic dietary exposure (highly exposed population) was estimated in the age class 'Infants' with estimates of 9.0 μg/kg bw per day and 8.2 μg/kg bw per day for Mpp75Aa1.1 and Vpb4Da2 proteins, respectively. The main contributor to the exposure in the dietary survey with the highest estimates would be corn flakes.

An ad hoc dietary exposure scenario was provided for consumers of pollen supplements under the assumption that these supplements might be made of pollen from MON 95275 maize. Since the expression levels of Mpp75Aa1.1 and Vpb4Da2 proteins were reported as below the LOQ for all pollen samples, the respective LOQs were used for the exposure estimations (see Table 1, Section 3.3.4). Consumption data on pollen supplements are available for few consumers across seven different European countries.[26] The low number of consumers available adds uncertainty to the exposure estimations which should be carefully interpreted, and only allows the estimation of dietary exposure for average consumers. The highest mean acute dietary exposure would be between 0.087 μg/kg bw per day for Mpp75Aa1.1 and 0.109 μg/kg bw per day for Vpb4Da2, in the elderly population. Similarly, the highest mean chronic dietary exposure in consumers of pollen supplements would be between 0.058 μg/kg bw per day for Mpp75Aa1.1 and 0.073 μg/kg bw per day for Vpb4Da2, also in the elderly population.

3.5.4.2 | *Animal dietary exposure*

Anticipated dietary exposure to Mpp75Aa1.1 and Vpb4Da2 proteins in maize MON 95275 was estimated across different animal species, as below described, assuming the consumption of maize products commonly entering the feed supply chain (i.e. maize grains, gluten feed, gluten meal and silage). A conservative scenario with 100% replacement of conventional maize products by the maize MON 95275 products was considered. Mean levels (dry weight) of the newly expressed proteins in grains and forage from maize MON 95275 used for animal dietary exposure are listed in Table 1 (Section 3.3.4).

Mean levels (dry weight) of the newly expressed proteins in maize gluten feed and gluten meal were calculated to be, respectively, 2.6- and 7.1-fold higher than in grain (as % of dry matter), based on adjusting factors that take into account the protein content in these feed materials relative to maize grain (OECD, 2002), and assuming that no protein is lost during their processing. The levels in forage were used as the silage values based on a conservative assumption that there is no protein loss.

The applicant estimated dietary exposure to Mpp75Aa1.1 and Vpb4Da2 proteins in maize MON 95275 via the consumption of maize grains, gluten feed and gluten meal in broiler and finishing pig and maize gluten feed, gluten meal and silage

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

[29] Example: 100 grams 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 ~0.91 μg of Vpb4Da2 per gram of maize bread as compared to the 1 μg/g reported as mean concentration in the maize grains.

in lactating dairy cow, based on default values for animal body weight, daily feed intake and inclusion rates (percentage) of maize feedstuffs in diets/rations, as provided for the EU by OECD (2009).

Estimated dietary exposure in the concerned animals is reported in Appendix D.

3.5.5 | Nutritional assessment of endogenous constituents

The intended trait of maize MON 95275 is insect protection against western corn rootworm, with no intention to alter nutritional parameters. Comparison of the composition of the maize MON 95275 with its conventional counterpart and the non-GM reference varieties did not identify differences that would require further safety assessment. From these data, the GMO Panel concludes that maize MON 95275 is nutritionally equivalent to its conventional counterpart and the non-GM reference varieties used.

3.5.6 | Post-market monitoring of GM food/feed

Maize MON 95275, as described in this application, does not raise any nutritional concern and is as safe as its conventional counterpart and the non-GM reference varieties tested. The GMO Panel concludes that, based on the information considered in its safety assessment, a post-market monitoring plan for food and feed is not necessary.

3.5.7 | Conclusions on the food/feed safety assessment

The proteins Mpp75Aa1.1, Vpb4Da2 and the DvSnf7.1 dsRNA and derived siRNAs newly expressed in maize MON 95275 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 MON 95275. The GMO Panel finds no evidence that the genetic modification impacts the overall safety of maize MON 95275. Based on the outcome of the comparative assessment and the nutritional assessment, the GMO Panel concludes that the consumption of maize MON 95275 does not represent any nutritional concern, in the context of the scope of this application. The GMO Panel concludes that maize MON 95275, as described in this application, is as safe as the conventional counterpart and the non-GM reference varieties tested, and no post-market monitoring of food/feed is considered necessary.

3.6 | Environmental risk assessment and monitoring plan[30]

3.6.1 | Environmental risk assessment

Considering the scope of application GMFF-2022-5890, which excludes cultivation, the environmental risk assessment (ERA) of maize MON 95275 mainly takes into account: (i) 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 (ii) the accidental release into the environment of GM material, including viable maize MON 95275 grains, during transportation and/or processing (EFSA GMO Panel, 2010a).

3.6.1.1 | *Persistence and invasiveness of the GM plant*

Maize is highly domesticated, not winter hardy in colder regions of Europe, and generally unable to survive in the environment without appropriate management. Survival is limited mainly by a combination of low competitiveness, absence of a dormancy phase and susceptibility to plant pathogens, herbivores and cold climate conditions (OECD, 2003), even though occasional feral GM maize plants may occur outside cultivation areas in the EU (e.g. Pascher, 2016). Field observations indicate that maize grains may survive and overwinter in some EU regions, resulting in volunteers in subsequent crops (e.g. Gruber et al., 2008; Palaudelmàs et al., 2009; Pascher, 2016). However, maize volunteers have been shown to grow weakly and flower asynchronously with the maize crop (Palaudelmàs et al., 2009). Thus, the establishment and survival of feral and volunteer maize in the EU is currently limited and transient.

It is unlikely that the intended trait of maize MON 95275 will provide a selective advantage to maize plants, except when they are infested by insect pests that are susceptible to the DvSnf7.1 dsRNA, or to the Mpp75Aa1.1 and/or Vpb4Da2 proteins. However, if this was to occur this fitness advantage will not allow the GM plant to overcome other biological and abiotic factors (described above) limiting plant's persistence and invasiveness. Therefore, the presence of the intended trait will not affect the persistence and invasiveness of the GM plant. The results of an additional study provided by the

[30]Dossier: Part II – Sections 5 and 6; additional information: 19/09/2023, 12/01/2024.

applicant on pollen viability and morphology (Appendix A) provided no evidence that maize MON 95275 has an increased risk of persistence and invasiveness than its conventional counterpart.

In conclusion, the GMO Panel considers it is very unlikely that maize MON 95275 will differ from conventional maize hybrid varieties in their ability to survive until subsequent seasons, or to establish occasional feral plants under European environmental conditions in case of accidental release into the environment of viable maize MON 95275 grains.

3.6.1.2 | *Potential for gene transfer*

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer (HGT) of DNA, or through vertical gene flow via cross-pollination from feral plants originating from spilled grains.

Plant-to-microorganism gene transfer

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

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

Homologous recombination is known to facilitate horizontal transfer of non-mobile, chromosomal DNA fragments to bacterial genomes. This requires the presence of at least two stretches of DNA sequences that are similar in the recombining DNA molecules. In the case of sequence identity with the transgene itself, recombination would result in gene replacement. In the case of identity with two or more regions flanking recombinant DNA, recombination could result in the insertion of additional DNA sequences in bacteria and thus confer the potential for new properties.

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

The bioinformatic analysis for event MON 95275 revealed no homology with known DNA sequences from bacteria which would facilitate homologous recombination.

In summary, there is no indication for an increased likelihood of horizontal transfer of DNA from maize MON 95275 to bacteria. Given the nature of the recombinant DNA, the GMO Panel identified no safety concern linked to an unlikely but theoretically possible HGT.

Plant-to-plant gene transfer

The potential for occasional feral maize MON 95275 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 (, 2022; Eastham & Sweet, 2002; EFSA, 2016; 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 (, 2022; EFSA, 2016; 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 3.5.1.1). Therefore, the likelihood/frequency of cross-pollination between occasional feral GM maize plants resulting from grain spillage, and weedy or cultivated *Zea* plants is considered extremely low (, 2022; EFSA, 2016). Even if cross-pollination would occur, the GMO Panel is of the opinion that environmental effects as a consequence of the spread of genes from occasional feral GM maize plants in Europe will not differ from that of conventional maize varieties for the reasons given in Section 3.6.1.1.

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

Taking the scope of application GMFF-2022-5890 into account (no cultivation), potential interactions of occasional feral maize MON 95275 plants arising from grain import spills with the target organisms are not considered a relevant issue.

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

The GMO Panel evaluated the potential hazards of the NEPs and the dsRNA and considered that the environmental exposure of non-target organisms to spilled GM maize material or occasional feral GM maize plants arising from spilled maize MON 95275 grains will be limited. Additionally, ingested dsRNA and proteins are typically degraded before entering the environment through faecal material of animals fed with maize MON 95275 (Dávalos et al., 2019; Harmon & Swanson, 2020; Miner-Williams et al., 2014; Mok & Urschel, 2020; Santos-Hernández et al., 2018; van Bruchem et al., 1985), and the data provided for the assessment of protein stability (see Section 3.5.1.2.3) supports that also the NEPs will be degraded. Given the limited environmental exposure, the GMO Panel considers that potential interactions of maize MON 95275 with non-target organisms do not raise any environmental safety concern. Interactions that may occur between the insecticidal proteins Mpp75Aa1.1 and Vpb4Da2 and dsRNA will not alter this conclusion.

3.6.1.5 | *Interactions with abiotic environment and biogeochemical cycles*

The GMO Panel evaluated the potential hazards of the NEPs and considered that the environmental exposure to spilled GM maize material or occasional feral GM maize plants arising from spilled maize MON 95275 grains will be limited. Additionally, ingested dsRNA and proteins are typically degraded before entering the environment through faecal material of animals fed with GM maize (Dávalos et al., 2019; Harmon & Swanson, 2020; Miner-Williams et al., 2014; Mok & Urschel, 2020; Santos-Hernández et al., 2018; van Bruchem et al., 1985), and the data provided for the assessment of protein stability (see Section 3.5.1.2.3) support that also the NEPs will be degraded. Given the limited environmental exposure, the GMO Panel considers that potential interactions of maize MON 95275 with the abiotic environment and biogeochemical cycles do not raise any environmental safety concern.

3.6.2 | Post-market environmental monitoring

The objectives of a post-market environmental monitoring (PMEM) plan, according to Annex VII of Directive 2001/18/EC, are: (i) 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 (ii) 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 MON 95275, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for maize MON 95275 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 MON 95275. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

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

The GMO Panel concludes that it is unlikely that maize MON 95275 would differ from conventional maize varieties in its ability to persist under European environmental conditions. Considering the scope of application GMFF-2022-5890, interactions of occasional feral maize MON 95275 plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from maize MON 95275 to bacteria does not indicate a safety concern. Therefore, considering the introduced trait, the outcome of the agronomic and phenotypic analysis and the routes and levels of exposure, the GMO Panel concludes that maize MON 95275 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 MON 95275.

4 | OVERALL CONCLUSIONS

The GMO Panel was asked to carry out a scientific assessment of maize MON 95275 for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003.

The molecular characterisation data establish that maize MON 95275 contains a single insert consisting of one copy of the *mpp75Aa1.1*, *vpb4Da2* and *DvSnf7* dsRNA expression cassettes. The quality of the sequencing methodology and data sets was assessed by the EFSA GMO Panel and is in compliance with the requirements listed in the EFSA Technical Note. Updated bioinformatics analyses of the sequences encoding the newly expressed proteins and other ORFs present within the insert or spanning the junctions between the insert and genomic DNA reveal similarity to known toxins, which was further assessed. The in planta RNAi off-target search, performed with the sequence of the *DvSnf7* 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 Mpp75Aa1.1 and Vpb4Da2 proteins is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of the plant and microbe-produced Mpp75Aa1.1 and Vpb4Da2 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 MON 95275 and its conventional counterpart needed further assessment. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the Mpp75Aa1.1 and Vpb4Da2 proteins as expressed in maize MON 95275, and finds no evidence that the genetic modification would change the overall allergenicity of maize MON 95275. In the context of this application, the consumption of food and feed from maize MON 95275 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that maize MON 95275 is as safe as the conventional counterpart and non-GM maize reference varieties tested, and no post-market monitoring of food/feed is considered necessary. The GMO Panel concludes that there is a very low likelihood of environmental effects resulting from the accidental release of material from maize MON 95275 into the environment. The PMEM plan and reporting intervals are in line with the intended uses of maize MON 95275. Based on the relevant publications identified through the literature searches, the GMO Panel does not identify any safety issues pertaining to the uses of maize MON 95275.

The GMO Panel concludes that maize MON 95275 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 23rd May 2022 concerning a request for authorization of the placing on the market of genetically modified maize MON 95275, submitted in accordance with Regulation (EC) No 1829/2003 by Bayer CropScience LP (EFSA Ref. GMFF-2022-589; EFSA-Q-2022-00330).
- The application was made valid on 29 August 2022.
- Additional Information (1) was requested on 22 September 2022.
- Additional information (1) was received on 07 November 2022.
- Additional information (2) was requested on 01 December 2022.
- Additional information (2) was received on 17 February 2023.
- Additional information (3) was requested on 04 April 2023.
- Additional information (3) was received on 12 May 2023.
- Additional information (4) was requested on 30 May 2023.
- Additional information (4) was received on 26 July 2023.
- Additional information (5) was requested on 19 July 2023.
- Additional information (5) was received on 19 September 2023.
- Additional information (6) was requested on 1 August 2023.
- Additional information (6) was received on 29 September 2023.
- Additional information (7) was requested on 13 October 2023.
- Additional information (7) was received on 15 December 2023.
- Additional information (8) was requested on 23 November 2023.
- Additional information (8) was received on 12 January 2024.
- Additional information (9) was requested on 09 February 2024.
- Additional information (9) was received on 28 March 2024.
- Additional information (10) was requested on 19 April 2024.
- Additional information (10) was received on 03 June 2024.
- Spontaneous information was received on 10 June 2024.

ABBREVIATIONS

ADF	acid detergent fibre
bp	base pair
bw	body weight
CaMV	cauliflower mosaic virus genome
CRM	comparative relative maturity

dsRNA	double-stranded ribonucleic acid
dw	dry weight
ELISA	enzyme-linked immunosorbent assay
ERA	environmental risk assessment
fw	fresh weight
GLP	good laboratory practice
GMO	genetically modified organism
HGT	horizontal gene transfer
HR	homologous recombination
JSA	junction sequence analysis
MS	mass spectrometry
NGS	next generation sequencing
NDF	neutral detergent fibre
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PCR	polymerase chain reaction
PMEM	post-market environmental monitoring
SDS–PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
T-DNA	transfer-deoxyribonucleic acid
UTR	untranslated region

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CONFLICT OF INTEREST

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

REQUESTOR

Competent Authority of the Netherlands

QUESTION NUMBER

EFSA-Q-2022-00330

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REFERENCES

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

Codex Alimentarius. (2009). Foods derived from modern biotechnology. In *Codex Alimentarius commission, joint FAO/ WHO food standards Programme*. https://www.fao.org/docrep/011/a1554e/a1554e00.htm

Collier, R. J., & Young, J. A. T. (2003). Anthrax toxin. *Annual Review of Cell and Developmental Biology*, *19*, 45–70.

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

Dávalos, A., Henriques, R., Latasa, M. J., Laparra, M., & Coca, M. (2019). Literature review of baseline information on non-coding RNA (ncRNA) to support the risk assessment of ncRNA-based genetically modified plants for food and feed. *EFSA Supporting Publication*, EN-1688. https://doi.org/10.2903/sp.efsa.6.EN-1688

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

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

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

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

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

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

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

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

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

EFSA (European Food Safety Authority). (2021a). Administrative guidance for the processing of applications for regulated products (update 2021). *EFSA Supporting Publications*, *18*(3), EN- 6471. https://doi.org/10.2903/sp.efsa.2021.EN-6471

EFSA (European Food Safety Authority). (2021b). Administrative guidance for the preparation of applications on genetically modified plants. *EFSA Supporting Publications*, *18*(3), EN-6473. https://doi.org/10.2903/sp.efsa.2021.EN-6473

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

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

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

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

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

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

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

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2017). Guidance on allergenicity assessment of genetically modified plants. *EFSA Journal*, *15*(5), 4862. https://doi.org/10.2903/j.efsa.2017.4862

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

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2018b). Scientific Opinion on the assessment of genetically modified maize MON 87411 for food and feed uses, import and processing, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2015-124). *EFSA Journal*, *16*(6), 5310. https://doi.org/10.2903/j.efsa.2018.5310

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

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

Foo, A. C. Y., & Mueller, G. A. (2021). Abundance and stability as common properties of allergens. *Frontiers in Allergy*, *2*, 769728. https://doi.org/10.3389/falgy.2021.769728

Friedlander, A. M. (1986). Marcophages are sensitive to anthrax lethal toxin through an acid-dependent process. *The Journal of Biological Chemistry*, *261*, 7123–7126.

Gruber, S., Colbach, N., Barbottin, A., & Pekrun, C. (2008). Post-harvest gene escape and approaches for minimizing it. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, *3*, 1–7.

Harmon, D. L., & Swanson, K. C. (2020). Review: Nutritional regulation of intestinal starch and protein assimilation in ruminants. *Animal*, *14*, S1–S28.

Heber, S., Borho, J., Stadler, N., Wondany, F., König, I., Michaelis, J., Papatheodorou, P., Barth, H., & Fellermann, M. (2023). The clostridium botulinum C2 toxin subunit C2IIa delivers enzymes with positively charged N-termini into the cytosol of target cells. *Toxins (Basel)*, *15*(6), 390. https://doi.org/10.3390/toxins15060390

Helm, R. M. (2001). Topic 5: Stability of Known Allergens (Digestive and Heat Stability). Report of a Joint FAO, WHO Expert Consultation on Allergenicity of Food Derived from Biotechnology, 22–25, January 2001. Food and Agriculture organisation of the United Nations (FAO), Italy, Rome.

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

Iurlina, M. O., & Fritz, R. (2005). Characterization of microorganisms in Argentinean honeys from different sources. *International Journal of Food Microbiology*, *105*, 297–304.

Kouadio, J.-L., Duff, S., Aikins, M., Zheng, M., Rydel, T., Chen, D., Bretsnyder, E., Xia, C., Zhang, J., Milligan, J., Evdokimov, A., Nageotte, J., Yin, Y., Moar, W., Giddings, K., Park, Y., Jerga, A., & Haas, J. (2021). Structural and functional characterization of Mpp75Aa1.1, a putative beta-pore forming protein from Brevibacillus laterosporus active against the western corn rootworm. *PLoS One*, *16*(10), e0258052. https://doi.org/10.1371/journal.pone.0258052

Kouadio, J.-L., Zheng, M., Aikins, M., Duda, D., Duff, S., Chen, D., Zhang, J., Milligan, J., Taylor, C., Mamanella, P., Rydel, T., Kessenich, C., Panosian, T., Yin, Y., Moar, W., Giddings, K., Park, Y., Jerga, A., & Haas, J. (2021). Structural and functional insights into the first bacillus thuringiensis vegetative insecticidal protein of the Vpb4 fold, active against western corn rootworm. *PLoS One*, *16*(12), e0260532. https://doi.org/10.1371/journal.pone.0260532

Le Corre, V., Siol, M., Vigouroux, Y., Tenaillon, M. I., & Dély, C. (2020). Adaptive introgression from maize has facilitated the establishment of teosinte as a noxious weed in Europe. *Proceedings of the National Academy of Sciences of the United States of America*, *117*, 25618–25627.
Lecoq, E., Holt, K., Janssens, J., Legris, G., Pleysier, A., Tinland, B., & Wandelt, C. (2007). General surveillance: Roles and responsibilities the industry view. *Journal für Verbraucherschutz Und Lebensmittelsicherheit-Journal of Consumer Protection and Food Safety*, *2*(S1), 25–28.
Li, Y., Tran, A. H., Danishefsky, S. J., & Tan, Z. (2019). Chemical biology of glycoproteins: From chemical synthesis to biological impact. *Methods in Enzymology*, *621*, 213–229.
Liu, X., Ma, A., Zhi, T., Hong, D., Chen, Z., Li, S., & Jia, Y. (2023). Dietary effect of Brevibacillus laterosporus S62-9 on chicken meat quality, amino acid profile, and volatile compounds. *Food*, *12*, 288. https://doi.org/10.3390/foods12020288
Liu, Y., Zai, X., Weng, G., Ma, X., & Deng, D. (2024). *Brevibacillus laterosporus*: A probiotic with important applications in crop and animal production. *Microorganisms*, *12*, 564. https://doi.org/10.3390/microorganisms12030564
Miner-Williams, W. M., Stevens, B. R., & Moughan, P. J. (2014). Are intact peptides absorbed from the healthy gut in the adult human? *Nutrition Research Reviews*, *27*, 308–329.
Moar, W. J., Evans, A. J., Kessenich, C. R., Baum, J. A., Bowen, D. J., Edrington, T. C., Haas, J. A., Kouadio, J.-L. K., Roberts, J. K., Silvanovich, A., Yin, Y., Weiner, B. E., Glenn, K. C., & Odegaard, M. L. (2017). The sequence, structural, and functional diversity within a protein family and implications for specificity and safety: The case for ETX_MTX2 insecticidal proteins. *Journal of Invertebrate Pathology*, *142*, 50–59.
Mok, C. H., & Urschel, K. L. (2020). Amino acid requirements in horses. *Asian-Australasian Journal of Animal Sciences*, *33*(5), 679–695.
Neumeyer, T., Schiffler, B., Maier, E., Lang, A. E., Aktories, K., & Benz, R. (2008). Clostridium botulinum C2 toxin. Identification of the binding site for chloroquine and related compounds and influence of the binding site on properties of the C2II channel. *The Journal of Biological Chemistry*, *283*(7), 3904–3914. https://doi.org/10.1074/jbc.M709807200
OECD (Organisation for Economic Co-operation and Development). (2002). Consensus document on compositional considerations for new varieties of maize (Zea Mays): key food and feed nutrients, anti-nutrients and secondary plant metabolites. Series on the safety of novel foods and feeds, No. 6-ENV/JM/MONO(2002)25.
OECD (Organisation for Economic Co-operation and Development). (2003). Consensus Document on the biology of *Zea mays subsp. mays* (Maize). Series on Harmonisation of Regulatory Oversight in Biotechnology (ENV/JM/MONO(2003)11), 27, 1–49.
OECD (Organisation for Economic Co-operation and Development). (2008). Test no. 407: Repeated dose 28-day oral toxicity study in rodents, OECD guidelines for the testing of chemicals, section 4. OECD Publishing. https://doi.org/10.1787/9789264070684-en
OECD (Organisation for Economic Co-operation and Development). (2009). Guidance document on overview of residue chemistry studies (as revised in 2009). Series on Testing and Assessment number 64 and Series on Pesticides number 32, OECD Environment, Health and Safety Publications, Paris, ENV/JM/MONO, 31, 93 pp.
OECD (Organisation for Economic Co-operation and Development). (2018). Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264070707-en
Paces, J., Nic, M., Novotny, T., & Svoboda, P. (2017). Literature review of baseline information to support the risk assessment of RNAi-based GM plants. *EFSA Supporting Publications*, EN-1246. https://doi.org/10.2903/sp.efsa.2017.en-1246
Palaudelmàs, M., Peñas, G., Melé, E., Serra, J., Salvia, J., Pla, M., Nadal, A., & Messeguer, J. (2009). Effect of volunteers on maize gene flow. *Transgenic Research*, *18*, 583–594.
Panda, A. K., Bisht, S. S., DeMondal, S., Kumar, N. S., Gurusubramanian, G., & Panigrahi, A. K. (2014). *Brevibacillus* as a biological tool: A short review. *Antonie Van Leeuwenhoek*, *105*, 623–639. https://doi.org/10.1007/s10482-013-0099-7
Pascher, K. (2016). Spread of volunteer and feral maize plants in Central Europe: Recent data from Austria. *Environmental Sciences Europe*, *28*, 30.
Polevoda, B., & Sherman, F. (2000). Na-terminal acetylation of eukaryotic proteins. *Journal of Biological Chemistry*, *275*, 36479–36482.
Santos-Hernández, M., Miralles, B., Amigo, L., & Recio, I. (2018). Intestinal signaling of proteins and digestion-derived products relevant to satiety. *Journal of Agricultural and Food Chemistry*, *66*, 10123–10131.
Sarkar, P. K., Hasenack, B., & Nout, M. J. R. (2002). Diversity and functionality of *bacillus* and related genera isolated from spontaneously fermented soybeans (Indian kinema) and locust beans (African soumbala). *International Journal of Food Microbiology*, *77*(3), 175–186. https://doi.org/10.1016/S0168-1605(02)00124-1
Schleberger, C., Hochmann, H., Barth, H., Aktories, K., & Schulz, G. E. (2006). Structure and action of the binary C2 toxin from clostridium botulinum. *Journal of Molecular Biology*, *364*(4), 705–715. https://doi.org/10.1016/j.jmb.2006.09.002
Sherer, K., Li, Y., Cui, X., & Eichacker, P. Q. (2007). Lethal and edema toxins in the pathogenesis of bacillus anthracis septic shock: Implications for therapy. *American Journal of Respiratory and Critical Care Medicine*, *175*(3), 211–221. https://doi.org/10.1164/rccm.200608-1239CP
Sun, J., Lang, A. E., Aktories, K., & Collier, R. J. (2008). Phenylalanine-427 of anthrax protective antigen functions in both pore formation and protein translocation. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 4346–4351. https://doi.org/10.1073/pnas.0800701105
Sys, C., Van Ranst, E., Debaveye, J., & Beernaert, F. (1993). Land evaluation. Part III: Crop requirements. Agricultural publication No. 7. Brussels, general Administration for Development Cooperation. 199 pp.
Trtikova, M., Lohn, A., Binimelis, R., Chapela, I., Oehen, B., Zemp, N., Widmer, A., & Hilbeck, A. (2017). Teosinte in Europe – Searching for the origin of a novel weed. *Scientific Reports*, *71*, 1560.
van Bruchem, J., Rouwers, S. M. G., Bangma, G. A., Lammers-Wienhoven, S. C. W., & van Adrichem, P. W. M. (1985). Digestion of proteins of varying degradability in sheep. 2. Amount and composition of the protein entering the small intestine. *Netherlands Journal of Agricultural Science*, *33*, 273–284.
Wang, C., Bean, G. J., Chen, C. J., Kessenich, C. R., Peng, J., Visconti, N. R., Milligan, J. S., Moore, R. G., Tan, J., Edrington, T. C., Li, B., Giddings, K. S., Bowen, D., Luo, J., Ciche, T., & Moar, W. J. (2022). Safety assessment of Mpp75Aa1.1, a new ETX_MTX2 protein from *Brevibacillus laterosporus* that controls western corn rootworm. *PLoS One*, *17*(9), e0274204.
Windels, P., Alcalde, E., Lecoq, E., Legris, G., Pleysier, A., Tinland, B., & Wandelt, C. (2008). General surveillance for import and processing: The EuropaBio approach. *Journal of Consumer Protection and Food Safety*, *3*(S2), 14–16.

SUPPORTING INFORMATION

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

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APPENDIX A

Additional studies

List of additional studies performed by or on behalf of the applicant with regard to the evaluation of the safety of maize MON 95275 for humans, animals or the environment.

Study identification	Title
M-787280-01-1	(2020) Pollen Viability and Morphology Evaluation of Maize MON 95275 Grown in a 2019 U.S. Field Trial

APPENDIX B

List of relevant publications identified by the applicant through literature searches (January 2012 to October 2023)

References

Edrington, T., Wang, R., McKinnon, L., Kessenich, C., Hodge-Bell, K., Li, W., Tan, J., Brown, G, Wang, C., Li, B., & Giddings, K. (2022). Food and feed safety of the bacillus thuringiensis derived protein Vpb4Da2, a novel protein for control of western corn rootworm. *PlosOne*, *17*, 1–18.

Food Standards Australia New Zealand. (2023). A1262. https://www.foodstandards.gov.au/code/applications/Documents/A1262_Approval%20Report.pdf

MAFF (Ministry of Agriculture, Forestry and Fisheries). (2022). Entry 124, コウチュウ目害虫抵抗性トウモロコシ(mpp75Aa1.1, vpb4Da2, DvSnf7.1, Zea mays subsp. mays (L.) Iltis) (MON95275, OECD-UI: MON-95275-7). https://www.maff.go.jp/j/syouan/nouan/carta/torikumi/attach/pdf/index-53.pdf

United States Food and Drug Administration. (2023). New Plant Variety Consultations. BNF No. 186. Corn. MON 95275, MON-95275-7. https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=NewPlantVarietyConsultations&id=MON-95275-7

Wang, C., Bean, G. J., Chen, C. J., Kessenich, C. R., Peng, J., Visconti, N. R., Milligan, J. S., Moore, R. G., Tan, J., Edrington, T. C., Li, B., Giddings, K. S., Bowen, D., Luo, J., Ciche T., & Moar, W. J. (2022). Safety assessment of Mpp75Aa1.1, a new ETX_MTX2 protein from Brevibacillus laterosporus that controls western corn rootworm. *Plos One*, *17*, 1–22.

APPENDIX C

Statistical analysis and statistically significant findings in the 28-day toxicity studies in mice and in the 90-day toxicity study in rats

C.1 | Statistical analysis of the 28-day toxicity study on *E. coli*-produced Mpp75Aa1.1 protein in mice

The following endpoints were statistically analysed: mortality, clinical signs, body weights, body weight gains, food consumption, functional observational battery, motor activity, ophthalmology, clinical pathology parameters (haematology, coagulation and serum chemistry), macroscopic necropsy findings, organ weights and microscopic examinations. 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 test diet groups (low, medium and high test substance Mpp75Aa1.1 groups) separately with the control group. Continuous endpoints were analysed, for each sex, with a linear model (factor: diet and for MA data: diet, time and interaction term 'dose-time'). For endpoints measured on a discrete scale, the comparisons were performed 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 the control diet group. Missing data were considered by the Panel and found not to have an impact on the results (Table C.1).

TABLE C.1 Statistically significant findings in the 28-day toxicity study on E. coli-produced Mpp75Aa1.1 protein in mice.

Statistically significant parameter/endpoint	Finding	GMO panel interpretation
Mortality	1 male (control, day 6). Lung and other lesions reported, indicating dosing error	Not related to MPP protein administration
Body weights	Increased (3%) in top dose male group on day 7 Decreased (4%) in female groups days 14 or 21	Small magnitude. Within normal variation. No impact on terminal body weights. Not an adverse effect of treatment
Body weight gain	Sporadic changes, increases and decreases in both sexes	Small magnitude. No clear dose response. Within normal variation. No impact on terminal body weights. Not an adverse effect of treatment
FoB, defaecation	Increased (200%) in low-dose female group	No dose response; high dose group value below controls. Within normal variation. Not an adverse effect of treatment
Haematocrit, haemoglobin, mean cell volume	Increased (5%) in low dose male group	Not adverse in isolation. Small magnitude. Not seen at top dose. Within normal variation. Not an adverse effect of treatment
Mean cell haemoglobin	Increased (5%) in mid- dose female group	Not adverse in isolation. Small magnitude. Not seen at top dose. Within normal variation. Not an adverse effect of treatment
Mean cell volume	Increased (5%) in mid- dose female group	Not adverse in isolation. Small magnitude. Not seen at top dose. Within normal variation. Not an adverse effect of treatment
Absolute reticulocyte count	Increased (30%) in top dose female group	Within normal variation only one value outside concurrent control range. Consistent with values for BSA group. Not an adverse effect of treatment

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

C.2 | Statistical analysis of the 28-day toxicity study on *E. coli*-produced Vpb4Da2 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 test diet groups (low, medium and high test substance Vpb4Da2 groups) separately with the control group. Continuous endpoints were analysed, for each sex, with a linear model (factor: diet and for MA data: diet, time and interaction term 'dose-time'). For endpoints measured on a discrete scale, the comparisons were performed 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 the control diet group. Missing data were considered by the Panel and found not to have an impact on the results (Table C.2).

TABLE C.2 Statistically significant findings in the 28-day toxicity study on E. coli-produced Vpb4Da2 protein in mice.

Statistically significant parameter/endpoint	Finding	GMO panel interpretation
Mortality	2 males (10 mg/kg bw/d, day 5; control, day 15). Lung lesions reported, indicating dosing error	Not related to Vp protein administration
ALT	Decreased (30%) in all female groups	Not adverse in isolation. No dose response. Control group has large SD. Consistent with values for BSA group. Not an adverse effect of treatment
Albumin	Decreased (6%) in top dose female group	Not adverse in isolation. Low magnitude. Within normal variation. Consistent with values for BSA group. Not an adverse effect of treatment
AST	Decreased (25%) in mid-dose female group	Not adverse in isolation. No dose response. Within normal variation. Consistent with values for BSA group. Not an adverse effect of treatment

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

C.3 | Statistical analysis of the 90-day study on maize MON 95275 in rats

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

The main statistical analysis compared protein (MON 95275) test diet groups (low/high) separately with the control group. The analysis was performed for sex-separated and pooled data at 5% level of significance. Continuous endpoints were analysed with a linear model (factor: diet group, in addition, sex and interaction 'diet-sex' for pooled analysis, whereas for Locomotor activity data: diet, time and the interaction term 'diet-time' for sex-separated analysis, in addition, sex and the interaction 'diet-sex' term for pooled analysis). Ranges from historical control data were provided to aid the assessment of statistically significant differences between the test and the control diet group. Missing data were considered by the Panel and found not to have an impact on the results (Table C.3).

TABLE C.3 Statistically significant findings in the 90-day toxicity study on maize MON 95275 in rats.

Statistically significant parameter/endpoint	Finding	GMO panel interpretation
Body weight gain	Decreased (10%) at the low dose, both sexes combined, week 0–1	Not seen at the top dose or at other time periods. No impact on terminal body weight. Not an adverse effect of treatment
Food consumption	Increases and decreases (10%) reported for a number of time periods in all test groups	Small magnitude. No impact on terminal body weight. Not an adverse effect of treatment
Rearing	Increased (10%) at the low dose, both sexes combined	Small magnitude. Not seen at the high dose. Within normal variation. Not an adverse effect of treatment
Total motor activity count	Increased (25%) at the low dose, both sexes combined	Not seen at the top dose. Within normal variation. Not an adverse effect of treatment
WBC count	Decreased (15%) at the top dose (both sexes combined)	Small magnitude. Within normal variation (only one top dose value was outside the control range). Not an adverse effect of treatment
Neutrophil count	Decreased (20%) at the top dose (both sexes combined)	Small magnitude. Within normal variation (all top dose values within the control range). Not an adverse effect of treatment
Potassium	Decreased (8%) in both female groups	Small magnitude. Within normal variation. Not an adverse effect of treatment
Aspartate transaminase	Decreased (15%) at the low dose, both sexes combined	Not seen at the top dose. Not adverse in isolation. Within normal variation. Not an adverse effect of treatment
Chloride	Decreased (1%) at the top dose (both sexes combined)	Small magnitude. Within normal variation (all top dose values within control range). Not an adverse effect of treatment
Sodium	Decreased (0.5%) at the top dose (both sexes combined)	Small magnitude. Within normal variation. Not an adverse effect of treatment
Triglycerides	Increased (20%) at the low dose, both sexes combined	Small magnitude. Within normal variation (all low dose values within control upper range). Not an adverse effect of treatment
Urinary pH	Increased (by 0.3 pH units to pH 7.0) at the low dose, both sexes combined	Small magnitude, urine remained approximately neutral. Not seen at the high dose. Within normal variation. Not an adverse effect of treatment

(Continues)

TABLE C.3 (Continued)

Statistically significant parameter/endpoint	Finding	GMO panel interpretation
T3	Increased (20%) at the top dose (both sexes combined)	Small magnitude. Within normal variation. No associated histopathology findings or changes in T4 or TSH. Not an adverse effect of treatment
Spleen weight (absolute and relative to body weight)	Decreased (10%) at the low dose, both sexes combined	Small magnitude. Not seen at the top dose. Within normal variation. No associated RBC or histopathology changes. Not an adverse effect of treatment
Prostate/seminal vesicle/ coagulating gland weight (absolute)	Decreased (10%) in both male groups	Small magnitude. Within normal variation (all top dose values within the control range). No associated histopathology changes. Not an adverse effect of treatment

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

APPENDIX D

Animal dietary exposure

TABLE D.1 Animal dietary exposure to mean concentrations of Mpp75Aa1.1 and Vpb4Da2 proteins in maize MON 95275 (µg/kg bw per day) based on the consumption of maize grains, gluten feed, gluten meal and silage.

Animal species BW (kg)/ total diet intake (kg dw)	Feed material	IR%	Mpp75Aa1.1	Vpb4Da2
Broiler 1.7/0.12	Grain	70	64	59
	Gluten feed	10	24	22
	Gluten meal	10	65	60
	Total	90	153	141
Finishing pig 100/3	Grain	70	27	25
	Gluten feed	20	20.4	19
	Gluten meal	10	28	25
	Total	100	75	69
Lactating dairy cow 650/25	Gluten feed	20[a]	26	24
	Gluten meal	20	71	65
	Silage	60	369	76
	Total	100	466	165

[a]For lactating dairy cow, the allocation of ingredient was based on first using the ingredient with the higher protein expression until 100% of daily intake is achieved. Thus, in this scenario, 100% of the dairy cow diet was achieved without maize grain, and using gluten feed at 20% of inclusion rate, although OECD, 2009 indicate 30%, which would exceed the 100% of the total diet.