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Assessment of genetically modified Maize MON 87429 for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2019-161)

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Abstract

Maize MON 87429 was developed to confer tolerance to dicamba, glufosinate, quizalofop and 2,4-D herbicides. The molecular characterisation data and bioinformatic analyses do not identify issues requiring food/feed safety assessment. None of the identified differences in the agronomic/phenotypic and compositional characteristics tested between maize MON 87429 and its conventional counterpart needs further assessment, except for the levels of phytic acid in grains, which do not raise nutritional and safety concerns. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the DMO, PAT, FT_T and CP4 EPSPS proteins as expressed in maize MON 87429. The GMO Panel finds no evidence that the genetic modification impacts the overall safety of maize MON 87429. In the context of this application, the consumption of food and feed from maize MON 87429 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that maize MON 87429 is as safe as the conventional counterpart and non-GM maize reference varieties tested, and no post-market monitoring of food/feed is considered necessary. In the case of accidental release of viable maize MON 87429 grains into the environment, this would not raise environmental safety concerns. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of maize MON 87429. The GMO Panel concludes that maize MON 87429, as described in this application, 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.

Keywords: GM, genetic engineering, maize (*Zea mays*), MON 87429, CP4 EPSPS, DMO, PAT, FT_T

Requestor: Competent Authority of The Netherlands

Question number: EFSA-Q-2019-00628

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Summary

Following the submission of application EFSA-GMO-NL-2019-161 under Regulation (EC) No 1829/2003 from Bayer Agriculture BV (referred to hereafter as 'the applicant'), the Panel on Genetically Modified Organisms of the European Food Safety Authority (referred to hereafter as 'GMO Panel') was asked to deliver a Scientific Opinion on the safety of genetically modified (GM) herbicide tolerant maize MON 87429 according to Regulation (EU) No 503/2013. The scope of application EFSA-GMO-NL-2019-161 is for import, processing, and food and feed uses within the European Union (EU) of maize MON 87429 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 87429 according to the scope of the application EFSA-GMO-NL-2019-161. The GMO Panel conducted the assessment of maize MON 87429 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 87429 contains a single insert consisting of one copy of the four (*pat*, *dmo*, *ft_t* and *cp4 epsps*) expression cassettes. The quality of the sequencing methodology and data sets was assessed by the GMO Panel and is in compliance to the requirements listed in the EFSA Technical Note, 2018. Updated bioinformatic analyses of the sequences encoding the newly expressed protein and open reading frames (ORFs) present within the insert or spanning the junctions between the insert and genomic DNA, do not raise any safety concerns. The stability of the inserted DNA and of the introduced trait is confirmed over several generations. The methodology used to quantify the levels of the DMO, PAT, FT_T and CP4 EPSPS proteins is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of plant- and microbe-produced DMO, PAT, FT_T and CP4 EPSPS proteins, indicate that these proteins are equivalent, and the microbe-produced proteins can be used in 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 87429 and its conventional counterpart needed further assessment, except for the levels of phytic acid in grains which do not raise nutritional and safety concerns. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the DMO, PAT, FT_T and CP4 EPSPS proteins as expressed in maize MON 87429. The GMO Panel finds no evidence that the genetic modification impacts the overall safety of maize MON 87429 food and feed. In the context of this application, the consumption of food and feed from maize MON 87429 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that maize MON 87429 is as safe as the conventional counterpart and non-GM maize reference varieties tested, and no post-market monitoring of food/feed is considered necessary.

Considering the introduced trait, the outcome of the agronomic and phenotypic analysis and the routes and levels of exposure, maize MON 87429 would not raise safety concerns in the case of accidental release of viable GM maize grains into the environment. The post-market environmental monitoring (PMEM) plan and reporting intervals are in line with the intended uses of maize MON 87429. 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 87429.

Based on the relevant publications identified through the literature searches, the GMO Panel does not identify any safety issues pertaining to the intended uses of maize MON 87429.

The GMO Panel concludes that maize MON 87429, as described in this application, 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.

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1. Introduction

The scope of the application EFSA-GMO-NL-2019-161 is for food and feed uses, import and processing of herbicide tolerant maize MON 87429 and does not include cultivation in the European Union (EU).

1.1. Background

On 2 October 2019, the European Food Safety Authority (EFSA) received from the Competent Authority of The Netherlands application EFSA-GMO-NL-2019-161 for authorisation of maize MON 87429 (Unique Identifier MON-87429-9), submitted by Bayer Agriculture BVBA (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003[1]. Following receipt of application EFSA-GMO-NL-2019-161, EFSA informed EU Member States and the European Commission, and made the application available to them. Simultaneously, EFSA published the summary of the application.[2]

EFSA checked the application for compliance with the relevant requirements of Regulation (EC) No 1829/2003 and Regulation (EC) No 503/2013[3], with the EFSA guidance documents, and, when needed, asked the applicant to supplement the initial application. On 16 January 2020, EFSA declared the application valid.

From the validity date, EFSA and its scientific Panel on Genetically Modified Organisms (hereafter referred to as 'GMO Panel') endeavoured to respect a time limit of 6 months to issue a scientific opinion on application EFSA-GMO-NL-2019-161. Such time limit was extended whenever EFSA and/or its GMO Panel requested supplementary information to the applicant. According to Regulation (EC) No 1829/2003, any supplementary information provided by the applicant during the risk assessment was made available to the EU Member States and EC (for further details, see the Section 'Documentation', below).

In accordance with Regulation (EC) No 1829/2003, EFSA consulted the nominated risk assessment bodies of EU Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC[4]. The EU Member States had 3 months to make their opinion known on application EFSA-GMO-NL-2019-161 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 87429 in the context of its scope as defined in application EFSA-GMO-NL-2019-161.

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

2. Data and methodologies

2.1. Data

The GMO Panel based its scientific risk assessment of maize MON 87429 on the valid application EFSA-GMO-NL-2019-161, additional information provided by the applicant during the risk assessment, relevant scientific comments submitted by the Member States and relevant peer-reviewed scientific publications.

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, pp. 1–23.
[2] Available online: https://open.efsa.europa.eu/study-inventory/EFSA-Q-2019-00628.
[3] Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. OJ L157, 8.6.2013, pp. 1–48.
[4] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 12.3.2001, pp. 1–38.
[5] Opinions of the nominated risk assessment bodies of EU Member States can be found at the Open EFSA Portal https://open.efsa.europa.eu/questions, querying the assigned Question Number.
[6] These particulars are available online at: https://open.efsa.europa.eu/study-inventory/EFSA-Q-2019-00628.

2.2. Methodologies

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

For this application, in the context of the contracts [OC/EFSA/GMO/2018/04], [OC/EFSA/GMO/2018/02], [EOI/EFSA/SCIENCE/2020/01-CT01GMO and -CT02GMO] and [OC/EFSA/GMO/2020/01], the contractors performed preparatory work for the evaluation of the applicant's literature search, of the methods applied for the statistical analysis, of the applicant's statistical analysis of the 90- and 28-day toxicity studies and of the completeness and quality of DNA sequencing information.

3. Assessment

3.1. Introduction

Maize MON 87429 was developed to confer tolerance to dicamba-, glufosinate-, quizalofop- and 2,4-D-based herbicides. Maize MON 87429 expresses DMO, PAT and FT_T proteins. In addition, maize MON 87429 expresses the CP4-EPSPS protein and utilises an endogenous maize RNAi regulatory element to suppress its expression in pollen (see Section 3.3.1). This results in a lack of viable pollen and thus male sterility when MON 87429 plants are exposed to glyphosate-containing herbicides at growth stages ranging from V8 to V13. This is part of a hybridisation system to be used in inbred lines to facilitate the hybrid seeds production. This is not considered an agronomic trait since the application of glyphosate outside the specific growth stages does not lead to male sterile plants but reduces plant yield compared to plants not expressing the same trait.

The GMO Panel previously assessed the DMO protein expressed in soybean MON 87708 and considered it safe with respect to potential effects on human and animal health and the environment (EFSA GMO Panel, 2013).

It should be noted that the assessment of herbicide residues relevant for this application is in the remit of the EFSA Plant Health & Pesticides Residues (PLANTS) Unit.

3.2. Systematic literature review[7]

The GMO Panel assessed the applicant's literature searches on maize MON 87429, which include a scoping review, according to the guidelines given in EFSA (2010, 2019).

A systematic review as referred to in Regulation (EU) No 503/2013 has not been provided in support to the risk assessment of application EFSA-GMO-NL-2019-161. 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 87429 at present. The GMO Panel considered the overall quality of the performed literature searches acceptable.

The literature searches identified one relevant peer-reviewed publication on maize MON 87429 from electronic databases, and four relevant records from the Internet pages of key organisations. The relevant publication is listed in Appendix A.

None of the relevant records identified through the literature searches reported information pointing to safety issues associated with maize MON 87429 relevant to the scope of this application.

3.3. Molecular characterisation[8]

3.3.1. Transformation process and vector constructs

MON 87429 was developed by *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*)-mediated transformation. Immature embryos of inbred maize line LH244 were co-cultured with a disarmed *A. tumefaciens* strain ABI containing the vector PV-ZMHT519224. The plasmid PV-ZMHT519224 used for the transformation carries *four* expression cassettes (*pat*, *dmo*, *ft_t* and *cp4 epsps*) between the right and the left border of the T-DNA, containing the following genetic elements:

[7] Dossier: Part II – Section 7; additional information provided: 14/6/2022.
[8] Dossier: Part II – Section 1.2; additional information provided: 19/8/2020, 28/8/2020, 9/6/2021, 7/10/2021, 15/11/2021.

– the *pat* expression cassette consists of promoter, 5′ UTR and intron sequences from a ubiquitin gene (*Ubq*) from *Erianthus ravennae*, the PAT coding sequence of the phosphinothricin *N*–acetyltransferase (PAT) gene of *Streptomyces viridochromogenes* and the 3′ UTR sequence of the fructose-bisphosphate aldolase (*Fba*) gene of *Setaria italica*;

– the *dmo* expression cassette consists of the promoter, 5′ UTR region and intron sequences of ubiquitin gene (*Ubq*) from *Coix lacryma-jobi*, the codon optimised sequence of the chloroplast transit peptide of the Albino and pale green 6 (*Apg6*) gene of *Arabidopsis thaliana*, the codon optimised coding sequence of the dicamba mono-oxygenase (DMO) gene of *Stenotrophomonas maltophilia* and the 3′ untranslated sequence of the *OsMt* gene of *Oryza sativa*;

– the *ft_t* expression cassette consists of the promoter, 5′ untranslated and intron sequences of the ubiquitin (*Ubq*) gene of *Arundo donax*, transit peptide containing sequence of the *Mdh* gene *of Arabidopsis thaliana*, the R-2,4-dichlorophenoxypropionate dioxygenase (*RdpA*) coding sequence of *Sphingobium herbicidovorans* that expresses the dioxygenase protein (FT_T) that confers tolerance to both FOPs (aryloxyphenoxypropionate (AOPP) acetyl coenzyme A carboxylase inhibitors) and 2,4-D herbicides, and the 3′ untranslated region of the no apical meristem (Nam) gene of *Oryza sativa*;

– the *cp4 epsps* expression cassette consists of the promoter and leader sequences of the 35 S RNA of cauliflower mosaic virus (CaMV), 5′ UTR sequence of the chlorophyll a/b-binding (CAB) gene of *Triticum aestivum*, intron and flanking UTR sequence of the *act1* gene of *Oryza sativa*, the chloroplast transit peptide containing sequence of the *ShkG* gene of *Arabidopsis thaliana*, the codon optimised coding sequence of the *aroA* gene of the *Agrobacterium* sp. strain CP4 that encodes for a EPSPS protein, modified partial 3′ UTR sequence of *Zea mays* cDNA (EU974548) that contains male tissue-specific siRNA target sequence and the 3′ UTR sequence of the glycine-rich RNA-binding protein (*Grp3*) gene of *Oryza sativa*.

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

3.3.2. Transgene constructs in the GM plant

Molecular characterisation of maize MON 87429 was performed by next generation sequencing (NGS), junction sequence analysis (JSA), polymerase chain reaction (PCR) and DNA sequence analysis to determine insert copy number, size and organisation of the inserted sequences and to confirm the absence of plasmid backbone sequences.

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

NGS and JSA of the whole genome demonstrated that maize MON 87429 contains a single insert, consisting of a single copy of the T-DNA in the same configuration as in the PV-ZMHT519224 transformation vector.

NGS/JSA also confirmed the absence of plasmid backbone sequences in the maize genome.

Sanger sequencing of PCR amplified fragments determined the nucleotide sequence of the entire maize MON 87429 locus consisting of 14,008 bp of the insert, 1,029 bp of 5′ and 1,031 bp of 3′ flanking regions. The Sanger analysis revealed that the insert in maize MON 87429 is characterised by a 29-bp insertion in the 5′ flanking, a 31-bp insertion in the 3′ flanking regions and a deletion of 54 bp as compared to the T-DNA sequence in the plasmid PV-ZMHT519224 used for the transformation.

MON 87429 CP4 EPSPS mRNA contains a 201 nucleotides region in the 3′ UTR which is recognised by the endogenous male tissue-specific siRNAs resulting in degradation of the CP4 EPSPS mRNA and reduced expression of the CP4 EPSPS protein in male tissues.

The possible interruption of known endogenous maize genes by the insertion in maize MON 87429 was evaluated by bioinformatic analyses of the pre-insertion locus and of the genomic sequences flanking the insert. The results of these analyses do not indicate the interruption of any known endogenous gene in maize MON 87429.

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

In addition, updated bioinformatic analyses of the amino acid sequences of the newly expressed PAT, DMO, FT_T and CP4 EPSPS proteins reveal no significant similarities to toxins and allergens. The updated bioinformatic analyses of the newly created open reading frames (ORFs) within the insert and spanning the junctions between the insert and genomic DNA do not indicate significant similarities to toxins and allergens.

To assess the possibility for horizontal gene transfer (HGT) by homologous recombination (HR), the applicant performed a sequence identity analysis for maize MON 87429 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 87429 expresses four new proteins: DMO, PAT, FT_T and CP4 EPSPS. Given the technical restraints in producing large enough quantities for safety testing from plants, these proteins were recombinantly produced in *Escherichia coli*. A set of biochemical methods was employed to demonstrate the equivalence between the MON 87429 and *E. coli* produced DMO, PAT, FT_T and CP4 EPSPS proteins. Purified proteins from these two sources were characterised and compared in terms of their biochemical, structural and functional properties.

3.3.3.1. DMO protein characterisation and equivalence

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and western blot analysis showed that plant- and microbe-produced DMO proteins had the expected molecular weight of ~ 38 kDa and were comparably immunoreactive to DMO protein-specific antibodies. Glycosylation detection analysis demonstrated that none of the DMO proteins were glycosylated. Amino acid sequence analysis of the plant-derived DMO protein by mass spectrometry (MS) methods showed that the protein matched the deduced sequence, as defined by the *dmo* gene. In addition, the MS data revealed that there was a variant of the plant-produced DMO that contained an additional cysteine residue at the N terminus resulting from the cleavage of the chloroplast transit peptide (CTP). Due to the difference of only one amino acid residue, the two variants were indistinguishable by SDS–PAGE or western blot analysis and could not be physically separated during protein purification. The MS data were consistent with the previously analysed microbe-produced DMO protein which was designed to also contain the additional cysteine residue at the N terminus. Functional equivalence was demonstrated by an *in vitro* assay which showed that plant and *E. coli*-derived DMO proteins (including those used in the 28-day toxicity study, see Section 3.5.3) had comparable enzymatic activity. High specificity of the DMO protein for dicamba has been previously demonstrated (EFSA GMO Panel, 2013).

3.3.3.2. PAT protein characterisation and equivalence

SDS–PAGE and western blot analysis showed that plant- and microbe-produced PAT proteins had the expected molecular weight of ~ 25 kDa and were comparably immunoreactive to PAT-specific antibodies. Glycosylation detection analysis demonstrated that none of the PAT proteins were glycosylated. Amino acid sequence analysis by MS showed that both proteins matched the deduced sequence as defined by the *pat* gene. In addition, the MS data showed that the N-terminal methionine of the plant-produced PAT protein was truncated. Such modifications are common in eukaryotic proteins (e.g. Poledova and Sherman, 2000) and have been previously assessed by the GMO Panel for newly expressed proteins (EFSA GMO Panel, 2017). Functional equivalence was demonstrated by a biochemical *in vitro* assay which showed that plant and microbe-produced PAT proteins had comparable activity for the intended herbicide.

3.3.3.3. FT_T protein characterisation and equivalence

SDS–PAGE and western blot analysis showed that both plant and microbe-produced FT_T proteins had the expected molecular weight of ~ 36 kDa and were comparably immunoreactive to FT_T protein-specific antibodies. Glycosylation detection analysis demonstrated that none of the FT_T proteins were glycosylated. Amino acid sequence of the plant-derived FT_T protein by MS methods showed that the protein matched the deduced sequence as defined by the *ft_t* gene. In addition, the MS data showed that the N-terminal methionine of the *E. coli*-produced FT_T was truncated and the plant-produced FT_T contained an additional cysteine residue at the N terminus resulting from the cleavage of the CTP included to target the FT_T protein to the chloroplast. Such modifications are common in eukaryotic proteins (e.g. Poledova and Sherman, 2000) and have been previously assessed by the GMO Panel for newly expressed proteins (EFSA GMO Panel, 2017). Functional equivalence was demonstrated by a biochemical *in vitro* assay which showed that plant and microbe-produced FT_T proteins had comparable enzymatic activity. The endogenous substrate specificity assessment

confirmed that the FT_T enzyme is unlikely to metabolise endogenous maize small molecules which share structural similarity with the intended substrates.

3.3.3.4. CP4 EPSPS protein characterisation and equivalence

SDS–PAGE and western blot analysis showed that both plant- and microbe-produced CP4 EPSPS proteins had the expected molecular weight of ~ 44 kDa and were comparably immunoreactive to CP4 EPSPS-specific antibodies. Glycosylation detection analysis demonstrated that none of the CP4 EPSPS proteins were glycosylated. Amino acid sequence analysis of the plant-produced CP4 EPSPS protein by MS and Edman degradation methods showed that the protein matched the deduced sequence as defined by the *cp4 epsps* gene. In addition, the MS data showed the presence of another two variants of the plant-produced CP4 EPSPS protein: the first with a truncated N-terminal methionine and the second variant showing the oxidation and acetylation of the N-terminal methionine. Such modifications are common in eukaryotic proteins (e.g. Poledova and Sherman, 2000) and have been previously assessed by the GMO Panel for newly expressed proteins (EFSA GMO Panel, 2017). Functional equivalence was demonstrated by a biochemical *in vitro* assay which showed that plant and microbe-produced CP4 EPSPS proteins had comparable enzymatic activity.

In tassel, the CP4 EPSPS mRNA is specifically recognised by the endogenous male tissue specific si-RNA, which induces its degradation specifically in this tissue without other significant effect.

The protein characterisation data comparing the biochemical, structural and functional properties of plant and microbe-produced DMO, PAT, FT_T and CP4 EPSPS proteins, indicate that these proteins are equivalent. Therefore, the GMO Panel accepts the use of the DMO, PAT, FT_T and CP4 EPSPS proteins produced in bacteria in the safety studies.

3.3.4. Information on the expression of the insert

Protein levels of PAT, DMO, FT_T and CP4 EPSPS were analysed by enzyme-linked immunosorbent assay (ELISA) in material harvested in a field trial across five locations in USA during the 2017 growing season. Samples analysed included grains (R6) and forage (R5) from plants treated and not treated with the intended herbicides.[9] The mean values, standard errors and ranges of protein expression levels in grains (n = 20) and forage (n = 20) of the PAT, DMO, FT_T and CP4 EPSPS proteins used to estimate human and animal dietary exposure (see Section 3.5.4) are reported in Table 1.

Table 1: Mean values (n = 20), standard errors and ranges of newly expressed proteins in grains [μg/g dry weight (dw) and μg/g fresh weight (fw)] and in forage [μg/g dry weight (dw)] from maize MON 87429

Tissues	Treatment with dicamba, glufosinate, quizalofop and 2,4-D			
	Not treated		Treated	
	μg/g dry weight (dw)	μg/g fresh weight (fw)[(d)]	μg/g dry weight (dw)	μg/g fresh weight (fw)[(d)]
Grains (R6)				
DMO	2.5[(a)] ± 0.19[(b)] (1.5–5.5)[(c)]	2.2 ± 0.17 (1.3–4.8)	2.4 ± 0.15 (1.3–3.6)	2.1 ± 0.13 (1.1–3.2)
PAT	0.97 ± 0.11 (0.38–2.6)	0.85 ± 0.10 (0.33–2.3)	0.86 ± 0.07 (0.32–1.5)	0.76 ± 0.06 (0.3–1.3)
FT_T	55 ± 5.2 (30–140)	48 ± 4.6 (26–123)	51 ± 4.0 (21–87)	45 ± 3.5 (18–77)
CP4 EPSPS	0.62 ± 0.03 (0.41–0.94)	0.55 ± 0.03 (0.36–0.83)	0.67 ± 0.03 (0.44–0.90)	0.59 ± 0.03 (0.4–0.79)
Forage (R5)				
DMO	23 ± 2.3 (7.9–54)		21 ± 1.6 (9.3–32)	
PAT	1.5 ± 0.11 (0.90–3.0)		1.3 ± 0.07 (0.72–1.9)	

[9] BBCH scale describes phenological stages. BBCH 85–87 corresponds to approximately R5 stage of maize development and BBCH 87–99 corresponds to R6.

Tissues	Treatment with dicamba, glufosinate, quizalofop and 2,4-D			
	Not treated		Treated	
	µg/g dry weight (dw)	µg/g fresh weight (fw)[d]	µg/g dry weight (dw)	µg/g fresh weight (fw)[d]
FT_T	110 ± 8.9 (57–240)		100 ± 5.5 (60–150)	
CP4 EPSPS	8.6 ± 0.76 (4.1–19)		8.2 ± 0.54 (4.3–12)	

(a): Mean value.
(b): Standard error.
(c): Range.
(d): Fresh weight values for DMO, PAT, FT_T and CP4 EPSPS proteins used to estimate human dietary exposure were calculated by multiplying the dry weight values by a dry weight correction factor of 0.88 to account for approximately 12% moisture content in the grains.

3.3.5. Inheritance and stability of inserted DNA

Genetic stability of MON 87429 insert was assessed by NGS/JSA from five generations (R3, R3F1, R4, R4F1 and R5) and qPCR-based segregation analysis from three generations (BC1, BC2, BC3). The results indicate that all the tested plants retained the single copy of the insert and flanking regions, which were stably inherited in subsequent generations.

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

3.3.6. Conclusion on molecular characterisation

The molecular characterisation data establish that maize MON 87429 contains a single insert consisting of one copy of the *pat*, *dmo*, *ft_t* and *cp4 epsps* expression cassettes. Bioinformatic analyses of the sequences encoding the newly expressed proteins and other ORFs within the insert or spanning the junctions between the insert and genomic DNA do not raise any safety concern. The stability of the inserted DNA and of the introduced traits is confirmed over several generations. The methodology used to quantify the levels of the PAT, DMO, FT_T and CP4 EPSPS proteins is considered adequate. The protein characterisation data comparing the structural, biochemical and functional properties of plant and microbe-produced PAT, DMO, FT_T and CP4 EPSPS proteins indicate that these proteins are equivalent, and those microbe-derived can be used in the safety studies.

3.4. Comparative analysis[10]

3.4.1. Overview of studies conducted for the comparative analysis

Application EFSA-GMO-NL-2019-161 presents data on agronomic and phenotypic characteristics, as well as on forage and grain composition of maize MON 87429 (Table 2).

Table 2: Main comparative analysis studies to characterise maize MON 87429 provided in the application EFSAGMONL2019161

Study focus	Study details	Comparator	Commercial non-GM reference varieties
Agronomic, phenotypic and compositional analysis	Field study, 8 sites in US[a]	LH244 × HCL617	19[b]

GM: genetically modified.
(a): The field trials were located in Audubon County, IA; Vermilion County, IL; Warren County, IL; Boone County, IN; Caswell County, NC; York County, NE; Miami County, OH; Lehigh County, PA.
(b): Agrigold A6267 (107); Agrigold A6472 (110); Agrigold A6574 (114); Dekalb DKC61-52 (111); Dekalb DKC62-06 (112); Dekalb DKC64-85 (114); Dekalb DKC65-18 (115); Golden Harvest G09C43 (109); Golden Harvest G12J11-A (112); Golden Harvest G15Z99 (115); Kruger K-0708 (108); Lewis 1,407 (107); Lewis 1,613 (113); LG Seeds LG2549 (109); LG Seeds LG2636 (114); Mycogen Seeds 2H721 (112); Mycogen Seeds MY09V40 (109); NH6769 (115); Stone 5,820 (108).

[10] Dossier: Part II – Section 1.3; additional information provided 10/4/2020, 18/12/2020 and 12/3/2021.

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 87429 exposed to the intended herbicides, maize MON 87429 not exposed to the intended herbicides (not treated), the comparator (hybrid LH244 × HCL617) and four commercial non-GM maize reference varieties (hereafter referred to as 'non-GM reference varieties').

The agronomic, phenotypic and compositional data were analysed as specified by EFSA GMO Panel (2010a, 2011a). This includes, for each of the two treatments of maize MON 87429, the application of a difference test (between the GM maize and its comparator) and of an equivalence test (between the GM maize and the set of 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

Maize event MON 87429 was obtained using the non-GM maize line LH244 as a recipient inbred line.

For the field trial study, the transformed inbred line LH244 was crossed with the non-GM inbred line HCL617 to produce the GM hybrid used to conduct the agronomic and phenotypic and the compositional assessment.

The comparator used in the field trials is the non-GM hybrid maize LH244 × HCL617 which has a similar genetic background as MON 87429 (as documented by the pedigree) and is, therefore, considered to be the conventional counterpart.

Maize MON 87429 and its conventional counterpart, both with a comparative relative maturity (CRM) of 111, are appropriate for growing in a range of environments across North America, where the comparative field trials were conducted.

Commercial non-GM reference varieties with a CRM ranging from 107 to 115 were selected by the applicant and, at each selected site, four of them were tested (see Table 2). On the basis of the information provided on CRM 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 the maize MON 87429 and of the conventional counterpart used in the 2017 field trials (see Table) were produced, harvested and stored under similar conditions. The seed lots were verified for their identity via event-specific PCR analysis. The grains were tested for their germination capacity under warm and cold temperature conditions. Germination capacity of the maize MON 87429 was compared with its conventional counterpart and the results[12] of these studies indicate that the seed germination of maize MON 87429 was not different than that of its comparator. The GMO Panel considers that the starting seed used as test material in the agronomic, phenotypic and compositional studies was of adequate quality.

3.4.3.3. Conclusion on suitability

The GMO Panel concludes that GM maize MON 87429, the conventional counterpart and the non-GM reference varieties were properly selected and are of adequate quality. Therefore, the test materials are considered appropriate for the comparative analysis.

3.4.4. Representativeness of the receiving environments

3.4.4.1. Selection of field trial sites

The selected field trial sites were located in commercial maize-growing regions of United States. The soil and climate characteristics of the selected fields were diverse,[13] corresponding to optimal, near-optimal and

[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] Hybrid maize MON 87429 showed a mean germination of 99% and 94% while the conventional counterpart showed a mean of 100% and 96% under warm and cold temperature conditions, respectively.

[13] Soil types of the field trials were silty clay loam, silt loam, loam and sandy; soil organic carbon ranged from 0.7% to 3.0%; pH ranged from 6.0 to 6.9; average temperatures and sum of precipitations during the usual crop growing season ranged from 16.0 to 20.3°C and from 555 to 848 mm, respectively.

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 sum of precipitations were provided on a weekly basis. No exceptional weather conditions were reported at any of the selected field trial sites. The GMO Panel considers that the meteorological data set falls within the range of climatic conditions normally occurring at these sites.

Management practices

The field trials included plots containing maize MON 87429, plots with the conventional counterpart and plots with non-GM reference varieties, mostly managed according to local agricultural practices. In addition, the field trials included plots containing the GM maize managed following the same agricultural practices, plus exposed at growth stage BBCH[14] 11–12 and BBCH 15–16 to a tank mixture containing glufosinate and quizalofop and to another containing dicamba and 2,4-D-based herbicides, respectively. Thinning was applied at some field trial sites to achieve a more homogeneous plant density across plots despite not considered a normal agricultural practice. In line with the scope of the application that excludes the use of glyphosate as an agronomic trait (see Section 3.1), the GMO Panel considers that the management practices, including sowing, harvesting and application of plant protection products, were acceptable for the selected receiving environments.

3.4.4.3. Conclusion on representativeness

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

3.4.5. Agronomic and phenotypic analysis

Agronomic and phenotypic endpoints tested under field conditions

Ten agronomic and phenotypic endpoints[15] plus information on biotic and abiotic stressors were collected from the field trials (see Table). The endpoint fruit count was not subjected to the statistical analysis because of limited variability in the data.

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

- For maize MON 87429 (not treated with the intended herbicides), no statistically significant differences were identified with the conventional counterpart. All the endpoints fell under equivalence category I.
- For maize MON 87429 (treated with the intended herbicides), the test of difference identified statistically significant differences with the conventional counterpart for early stand count, moisture and yield. All the endpoints fell under equivalence category I.

3.4.6. Compositional analysis

Forage and grain of maize MON 87429 harvested from the field trials (Table 2, Section 3.4.1) were analysed for 78 constituents (9 in forage and 69 in grain), including those recommended by OECD (2002). The statistical analysis was not applied to 15 grain constituents[16] because more than half of the samples were below the limit of quantification.

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

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

[16] Sodium, furfural, caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), myristoleic acid (C14:1), pentadecanoic acid (C15:0), 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) and arachidonic acid (C20:4).

The statistical analysis was applied to a total of 63 constituents (9 in forage[17] and 54 in grain[18]); a summary of the outcome of the test of difference and the test of equivalence is presented in Table 3:

For maize MON 87429 not treated with the intended herbicides, statistically significant differences in the comparison with the conventional counterpart were found for 19 endpoints (1 in forage and 18 in grains). All these endpoints fell under equivalence category I or II except for phytic acid in grain, for which the test of equivalence was not applied (because of the lack of variation among the non-GM reference varieties) (Table 4).

For maize MON 87429 treated with the intended herbicides, statistically significant differences in the comparison with the conventional counterpart were found for 18 endpoints (all in grain). All these endpoints fell under equivalence category I or II.

Table 3: Outcome of the comparative compositional analysis in forage and grain of maize MON 87429. The table shows the number of endpoints in each category

		Test of difference[a]			
		Not treated[c]		**Treated[c]**	
		Not different	**Significantly different**	**Not different**	**Significantly different**
Test of equivalence[b]	Category I/II	44	18[d]	41	18[d]
	Category III/IV	–	–	3[e]	–
	Not categorised	–	1[f]	1[g]	–
	Total endpoints	63		63	

(a): Comparison between maize MON 87429 and the conventional counterpart.
(b): Four different outcomes: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence). Not categorised means that the test of equivalence was not applied because of the lack of variation among the non-GM reference varieties.
(c): Treated/not treated with the intended herbicides.
(d): Endpoints with significant differences between maize MON 87429 and the conventional counterpart and falling under equivalence category I–II. For forage, not treated only: ash. Treated only: none. Both treated and not treated: none. For grains, not treated only: ADF, NDF, TDF, β-carotene, riboflavin and *p*-coumaric acid. Treated only: palmitic acid (C16:0), linoleic acid (C18:2), arachidic acid (C20:0), behenic acid (C22:0), calcium, phosphorus and magnesium. Both treated and not treated: total fat, carbohydrates by calculation, palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linolenic acid (C18:3), eicosenoic acid (C21:0), methionine, iron, α-tocopherol and raffinose.
(e): Levels of ADF, NDF and TDF in grain of not treated GM maize fell under equivalence category III, but no significant differences were identified between the GM maize and the conventional counterpart.
(f): Level of phytic acid in grain (not treated only) was not categorised for equivalence and a significant difference was identified between the GM maize and the conventional counterpart. Quantitative results are reported in Table 4.
(g): Level of phytic acid in grain (treated only) was not categorised for equivalence, but no significant differences were identified between the GM maize and the conventional counterpart.

The GMO Panel assessed all the significant differences between maize MON 87429 and the conventional counterpart, taking into account the potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties. For levels of phytic acid in grain, which were not categorised for equivalence, a significant difference was identified between the GM maize (not treated) and the conventional counterpart. Mean estimates for this endpoint are given in Table 4.

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

Table 4: Estimated means for the compositional endpoint in grain that is further assessed based on the results of the statistical analysis

Endpoint	Maize MON 87429		Comparator LH244 × HCL617	Non-GM reference varieties	
	Not treated[(a)]	Treated[(a)]		Mean	Equivalence limits
Phytic acid (% dw)	0.58*	0.63	0.68	0.64	–

dw: dry weight.
(a): Treated: treated with the intended herbicides; not treated: treated only with conventional herbicides (see Section 3.4.4.3).
For maize MON 87429, significantly different values are marked with an asterisk. For both treated and not treated GM, the endpoint could not be categorised for equivalence.

3.4.7. Conclusions on the comparative analysis

Considering the selection of test materials, the field trial sites and the associated management practices and the agronomic-phenotypic characterisation as an indicator of the overall field trial quality, the GMO Panel concludes that the field trials are appropriate to support the comparative analysis. Taking into account the natural variability observed for the set of non-GM reference varieties, the GMO Panel concludes that:

- None of the differences identified in agronomic and phenotypic characteristics tested between maize MON 87429 and the conventional counterpart needs further assessment regarding their potential environmental impact.
- None of the differences identified in forage and grain composition between maize MON 87429 and the conventional counterpart needs further assessment regarding food and feed safety except for levels of phytic acid in grain (not treated), which are further assessed in Section 3.5.

3.5. Food/feed safety assessment[19]

3.5.1. Effects of processing

Maize MON 87429 will undergo existing production processes used for conventional maize. No novel production process is envisaged. Based on the outcome of the comparative assessment, processing of the GM maize into food and feed products is not expected to result in products being different from those of conventional non-GM maize varieties.

3.5.2. Stability of the newly expressed protein

Protein stability is one of several relevant parameters to consider in the weight-of-evidence approach in protein safety assessment (EFSA GMO Panel, 2010b, 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 (Helm, 2001; Breiteneder and Mills, 2005; Foo and Mueller, 2021; Costa et al., 2022).

3.5.2.1. Effect of temperature and pH on the newly expressed protein

The effects of temperature and pH on PAT and CP4 EPSPS proteins expressed in MON 87429 were previously evaluated by the GMO Panel (EFSA GMO Panel, 2015a, 2018, 2021). The applicant provided additional studies on the DMO protein as expressed in MON 87429 that were consistent with the outcome of previous studies on other DMO proteins (EFSA GMO Panel, 2013). Briefly, samples of DMO protein from a microbial recombinant system were incubated for either 15 or 30 min at 25, 37, 55, 75 and 95°C followed by a functional activity assay and SDS–PAGE. The studies showed that DMO protein has no activity after incubation at temperatures ≥ 55°C. In relation to the effect of pH on the DMO protein, the molecular mass (~ 38 kDa) and immunoreactivity of the protein was unchanged at pH 1.2 and 7.5.

Furthermore, samples of FT_T protein from a microbial recombinant system were incubated for either 15 or 30 min at 4, 25, 37, 55, 75 and 95°C followed by a functional activity assay and

[19] Dossier: Part II – Sections 1.4, 1.5, 1.6, 2; additional information provided: 18/12/20, 12/3/21, 21/5/21, 28/3/22, 20/7/22.

SDS–PAGE. The studies showed that FT_T protein has no activity after incubation at temperatures ≥ 75°C. In relation to the effect of pH on the FT_T protein, the molecular mass (~ 35 kDa) and immunoreactivity of the protein was unchanged at pH 1.2 and 7.5.

3.5.2.2. *In vitro* protein degradation by proteolytic enzymes

In vitro protein degradation studies on proteins PAT and CP4 EPSPS expressed in MON 87429 were previously evaluated by the GMO Panel (EFSA GMO Panel, 2015a, 2018, 2021) The applicant provided additional studies on the DMO protein as expressed in MON 87429 that were consistent with the outcome of previous studies on other DMO proteins (EFSA GMO Panel, 2013). Briefly, the resistance to degradation by pepsin of the DMO protein was measured in solutions containing pepsin and the test protein at pH 1.2. The integrity of the test protein was analysed by gel electrophoresis followed by protein staining and Western analysis. No intact DMO protein was detected within 30 s of incubation. A short fragment of 3 kDa was present after 2 min of incubation but it was not observed after 5 min of incubation.

Furthermore, the applicant provided information on *in vitro* protein degradation of the FT_T protein. First, the applicant provided a resistance to degradation by pepsin of the FT_T protein from a microbial recombinant system in solutions at pH ~ 1.2. The integrity of the test protein in samples of the incubation mixture taken at various time points were analysed by SDS–PAGE gel electrophoresis followed by protein staining or by western blotting. The FT_T protein was degraded by pepsin within 0.5 min of incubation. Transient peptide fragments at ~ 4 kDa were observed at different time points by SDS–PAGE gel electrophoresis. Second, the resistance to degradation by pancreatin of the FT_T protein was also analysed in solutions at pH ~ 7.5. The FT_T protein was largely degraded after 5 min of incubation when analysed by western blotting. Finally, the applicant provided a voluntary study where the FT_T protein was subjected to a sequential digestion, pepsin followed by pancreatin. The transient peptide fragments seen in the pepsin analysis were degraded within 0.5 min of exposure to pancreatin when analysed by SDS–PAGE. The sequential addition of digestive enzymes (i.e. gastric digestion conditions followed by an intestinal *in vitro* digestion) has been proposed as part of several alternative protocols to the classical pepsin resistance test to more closely simulate (within the inherent limitations of *in vitro* models) the physiological conditions of gastrointestinal digestion (EFSA GMO Panel, 2021). This is in line with Codex Alimentarius which indicated that alternative *in vitro* digestion protocols may be used where adequate justification is provided (Codex Alimentarius, 2009).

3.5.3. Toxicology

3.5.3.1. Testing of the newly expressed protein

Four proteins (PAT, CP4 EPSPS, DMO and FT-T) are newly expressed in maize MON 87429. The potential for a functional interaction among them has been assessed with regard to human and animal health. These enzymatic proteins catalyse distinct biochemical reactions, acting on unrelated substrates (see Table 5). 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 87429.

Table 5: Intended effects of the NEPs in maize MON 87429

Protein	Intended effect in GM plant
PAT	The PAT protein confers tolerance to glufosinate-ammonium-based herbicides acting by acetylation of glufosinate-ammonium
CP4 EPSPS	The CP4 EPSPS protein confers tolerance to glyphosate-containing herbicides acting on the shikimic acid pathway for the biosynthesis of aromatic amino acids in plants, showing high substrate specificity.
DMO	The DMO protein confers tolerance to dicamba-containing herbicides acting by degrading the herbicide dicamba to the non-herbicidal compound 3,6-dichlorosalicylic acid and formaldehyde (catalysing the demethylation of dicamba)
FT-T	The FT_T protein confers tolerance to both FOPs (aryloxyphenoxypropionate (AOPP) acetyl coenzyme A carboxylase inhibitors) and 2,4-D herbicides. It is an alpha-ketoglutarate-dependent non-heme iron dioxygenase. The common reaction mechanism involves the formation of an iron-oxygen intermediate that targets specific substrates, in the case of FT_T protein leading to the degradation of synthetic auxin and aryloxyphenoxypropionate herbicides.

— *NEPs previously assessed*

The PAT and the CP4 EPSPS proteins were previously assessed by the GMO Panel in the context of other applications (EFSA, 2006, 2008, 2009b,c,d, 2011, 2012, 2013, 2014, 2015b, 2018) and no safety concerns for humans and animals (i.e. farmed and companion animals) were identified. Updated bioinformatics analyses revealed no similarities of the PAT and the CP4 EPSPS proteins with known toxins. Additional studies addressing acute toxicity of the PAT and CP4 EPSPS proteins were provided by the applicant and assessed by the GMO Panel (Appendix B). The GMO Panel is not aware of any new information that would change the previous conclusion on the safety of the PAT and the CP4 EPSPS proteins.

— *NEPs never assessed before*

The FT_T and the DMO proteins were never assessed by the GMO Panel, in the context of its previous Scientific Opinions, for the safety of humans and animals.

The GMO Panel has previously assessed other DMO proteins such as that expressed in soybean MON87708 (i.e. DMO and DMO+ 27) and no safety concerns for humans and animals were identified (EFSA GMO Panel, 2013). In that context, upon request of the GMO Panel to confirm the safety of the two DMO variants expressed in MON87708, a 28-day toxicity study in mice with the mixture of the two variants was assessed (EFSA GMO Panel, 2013). The GMO Panel noted that the DMO protein expressed in GM maize MON 87429 differs from that expressed in soybean MON 87708 for two amino acids, and for the presence of a different set of amino acid residues at the N terminus (i.e. DMO and DMO + 1 vs DMO and DMO + 27).

The GMO Panel assessed the safety profile of DMO and FT_T proteins in MON 87429, taking into account molecular characterisation and bioinformatic analyses (Section 3.3.2), the history of safe use for consumption of the newly expressed proteins, and the *in vitro* (Section 3.4.2) and *in vivo* studies.

Safety profile of DMO protein

i) Molecular characterisation

The plant-produced MON 87429 DMO protein has been extensively characterised and its equivalence to the microbial-produced protein was demonstrated (Section 3.3.3.1).

ii) Bioinformatic studies

No significant similarities of the DMO protein to toxins were identified (Section 3.3.2).

iii) History of safe use for consumption of the newly expressed protein

iii a. Information on the source organism

The DMO protein was originally derived from a *Stenotrophomonas maltophilia* strain found at the site of a dicamba manufacturing plant (Krueger et al., 1989). *S. maltophilia* is a Gram-negative bacterium, closely related to *Pseudomonas* species, that is ubiquitous in the environment. It is isolated from soil, water, animals and plants, where it is also found associated with the rhizosphere (Berg et al., 1999). In humans, *S. maltophilia* is an opportunistic pathogen that can develop multidrug resistance particularly among immune-compromised patients (Calza et al., 2003; Looney et al., 2009). However, *S. maltophilia* is not employed for the processing of foods or feeds and, therefore, dietary exposure to this bacterium or its products is to be considered incidental (Qureshi et al., 2005).

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

The DMO protein is a mono-oxygenase that catalyses in a substrate-specific reaction the *O*-demethylation of the herbicide dicamba, thus converting dicamba to the non-herbicidal reaction products 3,6-dichlorosalicylic acid and formaldehyde. This protein belongs to a family of enzymes known as Rieske non-heme iron oxygenases (Wang et al., 2016). Members of this family of enzymes display well-conserved secondary and tertiary structures, which confer the enzymatic function, but vary substantially at the level of their primary amino acid sequences (Ferraro et al., 2005). Proteins homologous to DMO are widespread in nature. Homologous proteins were identified in soil bacteria such as *Sphingobium* and *Sphingomonas* species, indicating a wide presence of such enzymes in soil microorganisms (Wang et al., 2016). However, as reported in the literature, even the closest known relative, i.e. vanillate *O*-demethylase from *Pseudomonas* and *Acinetobacter* species, display sequence identities to DMO protein of only 42% or less (D'Ordine et al., 2009). Also, the DMO proteins

expressed in maize MON 87429 differ in their amino acid sequences from the wild-type DMO protein from *S. maltophilia*.

iii c. Overall conclusion on the history of safe use

The source organism (*S. maltophilia*) is a spurious food or feed contaminant rather than being used for the production of foods or feeds, and conventional foods and feeds do not contain enzymes with high sequence homology to the DMO proteins. In addition, the DMO proteins of maize MON 87429 differ in their amino acid sequences from the wild-type DMO protein in *S. maltophilia*. The GMO Panel concludes that it is not possible to confirm a documented history for safe consumption of the DMO proteins.

iv) *In vitro* studies

The outcome of *in vitro* studies to characterise the stability of newly expressed DMO protein was described in Section 3.5.3.

v) *In vivo* studies[20]

The outcome of an acute toxicity study and of a 28-day study with the *E. coli*-produced MON 87429 DMO protein is described below.

Acute toxicity study

An acute toxicity study in CD-1 mice administered the *E. coli*-produced MON 87429 DMO protein by gavage at the dose of 1,000 mg/kg body weight (bw) showed no adverse effects.

28-day repeated dose toxicity study

For compliance with Article 6 of Regulation (EU) No 503/2013, the applicant spontaneously submitted a new 28-day repeated dose toxicity study in mice with the DMO protein which was evaluated by the GMO Panel.

The provided 28-day oral repeated-dose toxicity study on DMO was conducted in accordance with OECD TG 407 (2008) and the principles of Good Laboratory Practice (GLP).

Groups of Crl:CD-1(ICR) mice (20/sex per group), 7- to 8-week-old at the start of dosing, were allocated to six groups using a randomised complete block design. Groups were administered by oral gavage: the test substance (DMO protein) at targeted nominal doses of 1,000, 500, 100 and 10 mg/kg bw per day; 1,000 mg/kg bw per day of bovine serum albumin (BSA) (BSA control group) and the vehicle[21] (vehicle group). Mice were randomised to treatment groups (males and females separately) using a stratified randomization scheme designed to achieve similar group mean body weights (± 20% of the mean for each sex).

The test substance used in this study was produced by a recombinant system and contained about 96% DMO protein. The amino acid sequence analysis of the *E. coli*- produced DMO used in this 28-day toxicity study by mass fingerprint analysis matched the deduced sequence as defined by the *dmo* gene. This protein had the expected molecular weight and immunoreactivity to DMO 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.

The GMO Panel noted that animals were singly housed rather than paired, this is not unusual for mice. 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, Table C.1.

[20] Additional information: 14/6/2022.
[21] 20 mM potassium phosphate, pH 8.

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

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

Three deaths occurred in males during the study: one control animal was euthanised in poor condition on day 9; one top dose male was found dead on day 13 with no identified cause of death; one top dose male was found dead on day 7 with microscopic findings consistent with a dosing accident. None of the deaths are considered to be related to treatment with DMO protein.

No gross pathological findings related to the treatment with DMO protein were seen 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 histopathology findings related to the administration of the test diet compared to the control group.

The GMO Panel concludes that no adverse effects were observed in this 28-day mouse toxicity study on DMO protein at gavage doses up to 1,000 mg/kg bw per day.

vi) Conclusion on the toxicological profile of DMO protein

Based on the above information, the GMO Panel considers that there are no toxicological concerns for the DMO protein newly expressed in maize MON 87429.

Safety profile of FT_T protein

i) Molecular characterisation

The plant-produced FT_T protein has been extensively characterised and its equivalence to the microbial produced protein was demonstrated (Section 3.3.3.3).

ii) Bioinformatic studies

No significant similarities of FT_T protein to toxins were identified (Section 3.3.2).

iii) History of safe use for consumption of the newly expressed proteins

iii a. Information on the source organism

The FT_T protein is encoded by the *ft_t* gene, which is a modified version of the R-2,4-dichlorophenoxypropionate dioxygenase (*RdpA*) gene from the Gram-negative soil bacterium *Sphingobium herbicidovorans* (Horvath et al., 1990; Zipper et al., 1996), which is not known to be associated with human diseases. *Sphingobium* species have been found in the rhizosphere of plants and isolated as bacterial endophytes in maize (Rijavec et al., 2007). Representatives of the *Sphingomonas* group of bacteria, to which *Sphingobium* belongs, are exploited in the food industry for the biosynthesis of gelling agents (Fialho et al., 2008). They also serve for biodegradation and bioremediation purposes (Pozo et al., 2007; Zhao et al., 2017). However, *S. herbicidovorans* is not employed directly for the processing of foods or feeds and, therefore, dietary exposure to this bacterium or products thereof is to be considered incidental as a consequence of its presence in the environment.

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

The FT_T protein is an alpha-ketoglutarate-dependent non-heme iron dioxygenase. These enzymes have been identified in a broad range of organisms including bacteria, fungi, plants and vertebrates (Hausinger, 2004). Members of this superfamily share a common double-stranded, beta-helix protein

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

fold with three metal-binding amino acid residues coordinating the catalytic metal ion (Müller et al., 2006; Chekan et al., 2019). However, their overall protein sequence identity is limited (Schleinitz et al., 2004). The common reaction mechanism involves the formation of an iron-oxygen intermediate that targets specific substrates, in the case of FT_T protein leading to the degradation of synthetic auxin and aryloxyphenoxypropionate herbicides. The amino acid sequence of the FT_T protein expressed in maize MON 87429 shares 89% sequence identity with wild-type RdpA. A total of 30 amino acid substitutions throughout the protein sequence resulted in FT_T displaying an increased V_{max} and reduced K_m relative to RdpA. Also, compared to RdpA protein, these amino acid substitutions in FT_T confer a higher enzymatic activity at the typical summer temperatures encountered in maize growing areas.

iii c. Overall conclusion on the history of safe use

The source organism (*S. herbicidovorans*) is a spurious food or feed contaminant rather than being used for the production of foods or feeds, and conventional foods and feeds do not contain enzymes with high sequence homology to the FT_T protein. In addition, the FT_T protein of maize MON 87429 differs in the amino acid sequence from the wild-type FT_T protein in *S. herbicidovorans*. The GMO Panel concludes that it is not possible to confirm a documented history of safe consumption for the FT_T protein.

iv) *In vitro* studies

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

v) *In vivo* studies

The outcome of an acute tox study and of a 28-day study with the *E. coli*-produced MON 87429 FT_T protein is described below.

Acute toxicity study

An acute toxicity study in CD-1 mice administered the *E. coli*-produced MON 87429 FT_T protein by gavage at the dose of 2000 mg/kg bw showed no adverse effects.

28-day repeated dose toxicity study

For compliance with Article 6 of Regulation (EU) No 503/2013, the applicant spontaneously submitted a 28-day repeated dose toxicity studies in mice with the FT_T protein which was evaluated by the GMO Panel.

The 28-day oral repeated-dose toxicity study on FT_T provided was conducted in accordance with OECD TG 407 (2008) and the principles of Good Laboratory Practice (GLP).

Groups of Crl:CD-1(ICR) mice (20/sex per group), 7- to 8-week-old at the start of dosing, were allocated to five groups using a randomised complete block design. Groups were administered by oral gavage: the test substance (FT_T protein) at targeted nominal doses of 1,000, 300 or 100 mg/kg bw per day (high, medium and low FT_T protein groups); 1,000 mg/kg bw per day of bovine serum albumin (BSA) (BSA control group) and the vehicle[23] (vehicle group). Mice were randomised to treatment groups (males and females separately) using a stratified randomization scheme designed to achieve similar group mean body weights (± 20% of the mean for each sex).

The test substance used in this study was produced by a recombinant system and contained about 96% FT_T protein. The amino acid sequence analysis of the *E. coli*- produced FT_T used in this 28-day toxicity study by mass fingerprint analysis matched the deduced sequence as defined by the *ft_t* gene. This protein had the expected molecular weight and immunoreactivity to FT_T-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.

The GMO Panel noted that animals were singly housed rather than paired, this is not unusual for mice. Deviations to the protocol reported in the study were considered minor deviations with no impact on the study results.

[23] 0.5x Phosphate Buffered Saline (0.5 mM potassium phosphate monobasic, 1.5 mM sodium phosphate dibasic, 77.6 mM NaCl).

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, Table C.2.

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

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

Four animals were found dead or killed in poor conditions[24] and the cause of death remained undetermined. The applicant demonstrated the occurrence of unscheduled deaths of undetermined cause in historical controls relevant for this study. Considering the lack of pattern of these changes (different time points) and the lack of adverse findings in the other animals of the group, the GMO Panel considers these findings are not related with the treatment with the test protein. There were no reports of clinical signs associated with treatment.

No gross pathological findings related to the treatment with FT_T protein were seen 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 histopathology findings related to the administration of the test diet compared to the control group.

The GMO Panel concludes that no adverse effects were observed in this 28-day mouse toxicity study on FT_T protein at gavage doses up to 1,000 mg/kg bw per day.

vi Conclusion on the toxicological profile of FT_T protein

Based on the above information, the GMO Panel considers that there are no toxicological concerns for the FT_T protein newly expressed in maize MON 87429.

3.5.3.2. Testing of new constituents other than proteins

Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, no new constituents other than the newly expressed proteins have been identified in seed and forage from maize MON 84279. Therefore, no further food/feed safety assessment of components other than the newly expressed proteins is required.

3.5.3.3. Information on altered levels of food and feed constituents

Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, no altered levels of food/feed constituents have been identified in seed and forage maize MON 84279, except for levels of phytic acid in grain. These changes are considered not to represent a toxicological concern, considering the biological role of the affected constituent and the magnitude of the changes; therefore, no further toxicological assessment is needed. Further information on the relevance of these findings is provided in Section 3.5.6.

3.5.3.4. 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 84279 related to the stability and expression of the insert, and to modifications of toxicological concern in the composition of maize MON 84279 (see Sections 3.4.1, 3.4.2, and 3.4.3.3). Therefore, animal studies with food/feed derived from maize MON 84279 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 meal derived from maize MON 84279.

In this study, pair-housed Crl:CD(SD) rats (16 per sex-per group) were allocated to three groups using a randomised complete block design with 8 replication per sex. Groups were fed diets containing MON 84279 grains sprayed with the intended herbicides[25] at 50% and 33% of inclusion level (the

[24] One vehicle control female on day 1 of the study; one male at 1,000 mg/kg bw per day on day 27 and 2 females at 1,000 mg/kg bw on days 13 and 26, respectively.
[25] Glufosinate, quizalofop, dicamba and 2,4-D.

latter supplemented with 17% of the conventional counterpart maize) and the conventional counterpart LH244 + HCL617 (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.[26]

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 MON 84279 maize 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 Certified Rodent LabDiet® 5,002.

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 and a detailed description of the methodology and of statistically significant findings identified in rats is reported in Appendix C, Table C.3.

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

One female rat receiving the high-dose diet was found dead on day 51 of the study. No macroscopic or microscopic findings were noted at necropsy. This animal had not shown clinical signs. Therefore, the GMO Panel considered this intercurrent death incidental, not related to the test diet.

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 and 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 MON84279 grains up to/at 50% maize for 90 days.

3.5.4. Allergenicity

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

3.5.4.1. Assessment of allergenicity of the newly expressed proteins

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

[26] The panel noted that the control results for thyroid hormones were unusually low. The applicant responded (additional information 21/5/2021) that this was due to a technical issue in the laboratory. The Panel concluded that as there were no changes in thyroid weights or pathology, the thyroid was not affected by treatment with MON 84279.

The *pat*, *dmo*, *ft_t* and *cp4 epsps* genes originate from *S. viridochromogenes*, *S. maltophilia*, *S. herbicidovorans* and *Agrobacterium* sp. strain CP4, respectively, none of which are considered common allergenic sources.

Updated bioinformatic analyses of the amino acid sequences of the PAT, DMO, FT_T and CP4 EPSPS proteins, using the criterion of 35% identity in a sliding window of 80 amino acids, revealed no relevant similarities to known allergens. The studies on protein stability of the PAT, DMO, FT_T and CP4 EPSPS proteins have been described in Section 3.5.2. In addition, the GMO Panel did not find an indication that the newly expressed proteins PAT, DMO, FT_T and CP4 EPSPS at the levels expressed in maize MON 87429 might be adjuvants.

Furthermore, the applicant provided information on the safety of the PAT, DMO, FT_T and CP4 EPSPS proteins regarding their potential hazard to cause a celiac disease response.[27] For such assessment, the applicant followed the principles described in the EFSA GMO Panel guidance document (EFSA GMO Panel, 2017). The assessment of the DMO, FT_T and CP4 EPSPS proteins identified no perfect or relevant partial matches with known celiac disease peptide sequences. The assessment of the PAT protein revealed partial matches containing the Q/E-X1-P-X2 motif and required further investigations. Based on additional considerations on the position and nature of amino acids flanking the motif, such as the presence of two consecutive prolines and the charge and size of adjacent amino acids (EFSA GMO Panel, 2017), the relevant peptides containing the motif do not raise concern as they fail to mimic gluten sequences. Therefore, no indications of safety concerns were identified by the GMO Panel.

In the context of this application, the GMO Panel considers that there are no indications that the newly expressed PAT, DMO, FT_T and/or CP4 EPSPS proteins in maize MON 87429 may be allergenic.

3.5.4.2. Assessment of allergenicity of the whole GM plant or crop

The GMO Panel regularly reviews the available publications on food allergy to maize products. However, to date, maize is not considered a common allergenic food[28] (OECD, 2002). Therefore, the GMO Panel does not request experimental data to analyse the allergen repertoire of GM maize.

In the context of this application and considering the data from the molecular characterisation, the compositional analysis and the assessment of the newly expressed proteins (see Sections 3.3, 3.4 and 3.5), the GMO Panel identifies no indications of a potentially increased allergenicity of food and feed derived from maize MON 87429 with respect to that derived from the conventional counterpart and the non-GM reference varieties tested.

3.5.5. Dietary exposure assessment to new constituents

In line with Regulation (EU) No 503/2013 the applicant provided dietary exposure estimates to DMO, PAT, FT_T and CP4 EPSPS proteins newly expressed in MON 87429 maize. Dietary exposure was estimated based on protein expression levels reported in this application for MON 87429 maize treated with the intended herbicides, 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 DMO, PAT, FT_T and CP4 EPSPS proteins newly expressed in MON 87429 maize were derived from replicated field trials (four replicates from five locations, n = 20) in 2017 in the United States (see Table 2 in Section 3.4.1). Table 1 in Section 3.3.4. shows the protein expression levels used to estimate both human and animal dietary exposure.

3.5.5.1. Human dietary exposure

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

Mean protein expression values on fresh weight basis (see Table 1 in Section 3.3.4) are considered as the most adequate to estimate human dietary exposure (both acute and chronic) when working

[27] Technical dossier Section 1.5, additional information 18/12/2020 and 21/5/2021.

[28] 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.

with raw primary commodities that are commonly consumed as processed blended commodities (EFSA, 2019). Since no specific consumption data were available on commodities containing, consisting of, or obtained from MON 87429 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).[29] Corn oil was excluded from the assessment since no proteins are expected to be present in the oil.

For the acute dietary exposure estimations, the applicant directly assigned to processed commodities the mean value reported for the concentration of DMO, PAT, FT_T and CP4 EPSPS proteins in MON 87429 maize grains. Overall, this is a conservative approach as neither recipes nor the effect of processing on the final concentration of newly expressed proteins are considered, except for corn oil which is eventually excluded from the exposure estimations. Summary statistics of consumption from the EFSA consumption database were used.[30] Acute dietary exposure in high consumers within each dietary survey and age class was estimated by summing the exposure derived from the 95th percentile consumption for the dominant food commodity[29] among consumers only and those exposures derived from the mean consumption of the remaining food categories in the total population (EFSA, 2015). The highest acute dietary exposure was estimated in the age class 'Infants' with exposure estimates of 504, 23.5, 9.0 and 6.7 mg/kg bw per day for FT_T, DMO, PAT and CP4 EPSPS proteins, respectively.

The GMO Panel estimated chronic dietary exposure to DMO, PAT, FT_T and CP4 EPSPS proteins. Individual consumption data of the relevant food commodities were retrieved from the EFSA Consumption Database, using dietary surveys with at least 2 days consumption and covering a total of 22 European countries.[31] 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.[32] No losses in the newly expressed proteins during processing were considered, except for certain commodities excluded from the exposure estimations (maize oil, corn starch, corn syrup). The 95th percentile chronic exposure (highly exposed population) was derived from the distribution of the individual dietary exposure estimates within each dietary survey and age class. The highest chronic dietary exposure was estimated in the age class 'Infants' with exposure estimates of 197, 9.2, 3.5 and 2.6 mg/kg bw per day for FT-T, DMO, PAT and CP4 EPSPS proteins, respectively. The main contributor to the exposure in the dietary survey with the highest estimates was sweet corn.

Consumption data on pollen supplements are available for few consumers across eight different European countries in the EFSA consumption database.[33] Still, when assessing the dietary exposure to DMO, PAT, FT_T and CP4 EPSPS via the consumption of pollen supplements, the particularities of MON 87429 maize should be considered (see Section 3.1). The intended use of MON 87429 maize is for hybrid seed production with MON 87429 to be used as female plant following its treatment with glyphosate. This treatment at specific growth stages results in non-viable pollen. This pollen is expected not to contain the CP4 EPSPS protein while the other NEPs might be present but at negligible amounts.

The assumption that MON 87429 maize is imported as single event F1 for food and feed uses (see scope of the application) implies that no treatment with glyphosate would be applied. This would result in MON 87429 maize producing viable pollen containing DMO, PAT and FT_T newly expressed proteins. Therefore, humans could be exposed to newly expressed proteins via the consumption of pollen from MON 87429 maize, with the exception of CP4 EPSPS protein. However, since no data on the presence of newly expressed proteins in pollen were available, the potential dietary exposure to DMO, PAT and FT_T proteins from the consumption of pollen supplements under this scenario could not be estimated.

[29] Dominant food commodity refers to the food that will lead to the highest exposure among all consumed foods.
[30] Summary statistics from the EFSA Comprehensive European Food Consumption Database accessed in June 2019 (http://www.efsa.europa.eu/en/applications/gmo/tools).
[31] Austria, Belgium, Bulgaria, Cyprus, the Czech Republic, Germany, Denmark, Estonia, Finland, France, Greece, Croatia, Hungary, Ireland, Italy, Latvia, the Netherlands, Portugal, Spain, Romania, Slovenia and Sweden. Data accessed: March 2021.
[32] Example: 100 g of maize bread are made with approximately 74 g of maize flour, and a reverse yield factor of 1.22 from the conversion of maize grains into flour is used. This results in 41 μg of FT_T per gram of maize bread as compared to the 45 μg/g reported in the maize grains.
[33] https://www.efsa.europa.eu/en/food-consumption/comprehensive-database. Data accessed: March 2021.

3.5.5.2. Animal dietary exposure

Dietary exposure to DMO, PAT, FT_T and CP4 EPSPS proteins in maize MON 87429 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 forage/silage). A conservative scenario with 100% replacement of conventional maize products by the maize MON 87429 products was considered. Mean levels (dry weight) of the newly expressed proteins in grains and forage from the maize MON 87429 treated with the intended herbicide used for 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, 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 production/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 DMO, PAT, FT_T and CP4 EPSPS proteins via the consumption of maize grains, gluten feed and gluten meal in broiler and finishing pig and maize gluten feed, gluten meal and silage 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, Table D.1.

3.5.6. Nutritional assessment of GM food and feed/endogenous constituents

The intended traits of maize MON 87429 are herbicide tolerance with no intention to alter nutritional parameters. However, levels of phytic acid in grains (not treated) were significantly different from its conventional counterpart and could not be categorised for equivalence (Section 3.4.6). Phytic acid is the primary storage form of phosphorus in seeds (as much as 60%–75% of the total phosphorus in the grains is bound to phytic acid, OECD, 2002), and protects the seed against oxidative stress (D'Ordine et al., 2009).

The biological relevance of this compound, the role of maize as a contributor to its total intake and the magnitude and direction of the observed changes were considered during the nutritional assessment.

3.5.6.1. Human nutrition

Phytic acid (myo-inositol 1, 2, 3, 4, 5, 6-hexakis-dihydrogen phosphate) is typically considered as an antinutrient that reduces mineral bioavailability. In maize, phytic acid is mainly located in the germ (88%) (Feizollahi et al., 2021). In the context of human nutrition, some processing methods (e.g. nixtamalisation,[34] fermentation) will decrease the levels of phytic acid increasing the bioavailability of different nutrients present in the grain (minerals, vitamins, etc.). Considering that the levels of phytic acid in maize MON 87429 are lower than those in its conventional counterpart, the GMO Panel concludes that the level of phytic acid in maize MON 87429 does not represent a nutritional concern.

3.5.6.2. Animal nutrition

The decrease in the levels of phytic acid reported in maize MON 87429 grains does not represent a nutritional concern for farmed and companion animals. Phytic acid is a compound found in most plant-based feed ingredients which binds with vital nutrients like minerals, vitamins and amino acids, reducing their availability to the animals (anti-nutritional factor). Phosphorus is primarily stored in the form of phytates, phytic acid bound to a mineral, in plant seeds, that makes phosphorus poorly digestible. The phosphorus digestibility of maize in swine is around 30%, while when adding phytase it raises to 50%–60%, allowing a reduction of inorganic sources of phosphorus to satisfy nutritional requirements. Rumen bacteria can hydrolyse partially phytates making phosphorus available.

3.5.7. Post-market monitoring of GM food/feed

The GMO Panel concluded that maize MON 87429, as described in this application, does not raise any nutritional concern and is as safe as the conventional counterpart and the non-GM reference

[34] Maize processing method which involves boiling the maize in water containing lime (calcium hydroxide) at a concentration range of 1%–5% (Gomez et al., 1991).

varieties tested. Therefore, the GMO Panel considers that post-market monitoring of food and feed from this GM maize, as described in this application, is not necessary.

3.5.8. Conclusion on the food and feed safety assessment

The proteins DMO, PAT, FT_T and CP4 EPSPS newly expressed in maize MON 87429 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 87429. The GMO Panel found no evidence that the genetic modification impacts the overall safety of maize MON 87429. Based on the outcome of the comparative assessment and the nutritional assessment, the GMO Panel concludes that the consumption of maize MON 87429 does not represent any nutritional concern, in the context of the scope of this application. The GMO Panel concludes that maize MON 87429, 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[35]

3.6.1. Environmental risk assessment

Considering the scope of application EFSA-GMO-NL-2019-161, which excludes cultivation, the environmental risk assessment (ERA) of maize MON 87429 mainly takes into account: (1) the exposure of microorganisms to recombinant DNA in the gastrointestinal tract of animals fed GM material and of microorganisms present in environments exposed to faecal material of these animals (manure and faeces); and (2) the accidental release into the environment of viable maize MON 87429 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. Occasional feral GM maize plants may occur outside cultivation areas in the EU (e.g. Pascher, 2016), but survival is limited mainly by a combination of low competitiveness, absence of a dormancy phase and susceptibility to plant pathogens, herbivores and cold climate conditions (OECD, 2002). Field observations indicate that maize grains may survive and overwinter in some EU regions, resulting in volunteers in subsequent crops (e.g. Gruber et al., 2008; Palaudelmàs et al., 2009; Pascher, 2016). However, maize volunteers have been shown to grow weakly and flower asynchronously with the maize crop (Palaudelmàs et al., 2009). Thus, the establishment and survival of feral and volunteer maize in the EU is currently limited and transient.

It is unlikely that the intended traits of maize MON 87429 will provide a selective advantage to maize plants, except when they are exposed to glufosinate ammonium and/or -quizalofop and/or 2,4-D- and/or dicamba containing herbicides. However, this fitness advantage will not allow the GM plant to overcome other biological and abiotic factors (described above), limiting plant's persistence and invasiveness outside the confines of a managed system. In addition, maize MON 87429 expresses the CP4 EPSPS protein (see Section 3.1). When MON 87429 plants are exposed to glyphosate-containing herbicides at growth stages ranging from V8 to V13, this will result in a lack of viable pollen while application of glyphosate outside these specific growth stages does not lead to male sterile plants but reduces plant yield compared to plants not expressing the same trait. Therefore, the traits maize MON 87429 expresses will not affect the persistence and invasiveness of the GM plant.

In conclusion, the GMO Panel considers that maize MON 87429 will be equivalent to 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 87429 grains.

[35] Dossier: Part II – Section 5; additional information provided 15/11/2021.

3.6.1.2. Potential for gene transfer

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

Plant-to-microorganism gene transfer

Genomic DNA can be a component of food and feed products derived from maize. It is well documented that such DNA becomes substantially degraded during processing and digestion in the human or animal gastrointestinal tract. However, bacteria in the digestive tract of humans and 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, 2009a).

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 (Hülter and Wackernagel, 2008; EFSA, 2009a). 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 87429 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 87429 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 87429 plants originating from grain import spills to transfer recombinant DNA to sexually compatible plants and the environmental consequences of this transfer were considered.

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

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

The potential of spilled maize grains to establish, grow and produce pollen is extremely low and transient (see Section 3.6.1.1). Therefore, the likelihood/frequency of cross-pollination between occasional feral GM maize plants resulting from grain spillage, and weedy or cultivated *Zea* plants is considered extremely low (EFSA, 2016, 2022). Even if cross-pollination would occur, the GMO Panel is of the opinion that environmental effects as a consequence of the spread of genes from occasional feral GM maize plants in Europe will not differ from that of conventional maize varieties for the reasons given in Section 3.6.1.2, even if exposed to the intended herbicides.

3.6.1.3. Interactions of the GM plant with target organisms

Taking the scope of application EFSA-GMO-NL-2019-161 (no cultivation) and the absence of target organisms into account, potential interactions of occasional feral maize MON 87429 plants arising from grain import spills with target organisms are not considered a relevant issue.

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

Given that environmental exposure of non-target organisms to spilled GM grains or occasional feral GM maize plants arising from spilled maize MON 87429 grains is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM maize, the GMO Panel considers that potential interactions of maize MON 87429 with non-target organisms do not raise any environmental safety concern.

3.6.1.5. Interactions of the GM plant with abiotic environment and biogeochemical cycles

Given that environmental exposure to spilled grains or occasional feral maize MON 87429 plants arising from grain import spills is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM maize, the GMO Panel considers that potential interactions with the abiotic environment and biogeochemical cycles do not raise any environmental safety concern.

3.6.2. Post-market environmental monitoring[36]

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

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

As the ERA did not identify potential adverse environmental effects from maize MON 87429, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for maize MON 87429 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 87429. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

3.6.3. Conclusion on the environmental risk assessment and monitoring plan

The GMO Panel concludes that it is unlikely that maize MON 87429 would differ from conventional maize varieties in its ability to persist under European environmental conditions. Considering the scope of application EFSA-GMO-NL-2019-161, interactions of occasional feral maize MON 87429 plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from maize MON 87429 to bacteria does not indicate a safety concern. Therefore, considering the introduced traits, the outcome of the agronomic and phenotypic analysis, and the routes and levels of exposure, the GMO Panel concludes that maize MON 87429 would not raise safety concerns in the event of accidental release of viable GM maize grains into the environment.

The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of maize MON 87429.

4. Overall conclusions

The GMO Panel was asked to carry out a scientific assessment of maize MON 87429 for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003. The molecular characterisation data establish that the GM maize MON 87429 contains a single insert consisting of one copy of the (*pat*, *dmo*, *ft_t* and *cp4 epsps*) expression cassettes. The quality of the sequencing methodology and data sets was assessed by the EFSA GMO Panel and is in compliance to the

[36] Dossier: Part II – Section 6; additional information provided 14/4/2022.

requirements listed in the EFSA Technical Note, 2018. Updated bioinformatic analyses of the sequences encoding the newly expressed proteins and other ORFs present within the insert or spanning the junctions between the insert and genomic DNA, do not raise any safety concerns. The stability of the inserted DNA and of the introduced traits is confirmed over several generations. The methodology used to quantify the levels of the DMO, PAT, FT_T and CP4 EPSPS proteins is considered adequate. The proteins characterisation data comparing the biochemical, structural and functional properties of plant- and microbe-produced DMO, PAT, FT_T and CP4 EPSPS proteins indicate that they are equivalent and that the microbe-produced proteins can be used in 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 87429 and its conventional counterpart needed further assessment, except for the levels of phytic acid which do not raise nutritional and safety concerns. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of DMO, PAT, FT_T and CP4 EPSPS proteins as expressed in maize MON 87429. The GMO Panel finds no evidence that the genetic modification impacts the overall safety of maize MON 87429. In the context of this application, the consumption of food and feed from maize MON 87429 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that maize MON 87429 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 viable grains from maize MON 87429 into the environment. The PMEM plan and reporting intervals are in line with the intended uses of maize MON 87429. Based on the relevant records retrieved through the literature searches, the GMO Panel does not identify any safety issues pertaining to the intended uses of maize MON 87429.

In conclusion, the GMO Panel considers that maize MON 87429, as described in this application, 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 2nd October 2019 concerning a request for authorization of the placing on the market of genetically modified maize MON 87429 submitted in accordance with Regulation (EC) No 1829/2003 by Bayer Agriculture BVBA (EFSA-GMO-NL-2019-161; EFSA-Q-2019-00628)
- The application was made valid on 16 January 2020
- Additional Information (Clock 1) was requested on 7 February 2020
- Additional Information (Clock 1) was received on 10 April 2020
- Additional Information (Clock 2) was requested on 29 April 2020
- Additional Information (Clock 2) was received on 28 August 2020
- Additional Information (Clock 3) was requested on 19 June 2020
- Additional Information (Clock 3) was received on 19 August 2020
- Additional Information (Clock 4) was requested on 29 October 2020
- Additional Information (Clock 4) was received on 18 December 2020
- Additional Information (Clock 5) was requested on 18 January 2021
- Additional Information (Clock 5) was received on 12 March 2021
- Additional Information (Clock 6) was requested on 26 February 2021
- Additional Information (Clock 6) was received on 9 June 2021
- Additional Information (Clock 7) was requested on 17 March 2021
- Additional Information (Clock 7) was received on 21 May 2021
- Additional Information (Clock 8) was requested on 14 June 2021
- Additional Information (Clock 8) was received on 7 October 2021 partial, 28 March 2022 complete
- Additional Information (Clock 9) was requested on 30 September 2021
- Additional Information (Clock 9) was received on 15 November 2021
- Additional Information (Clock 10) was requested on 14 April 2022
- Additional Information (Clock 10) was received on 14 June 2022

- Supplementary information was provided on voluntary basis on 24 February 2020
- 10 April 2020; 2 September 2020; 30 October 2020; 27 January 2021; 14 June 2022
- Additional Information (Clock 11) was requested on 18 July 2022
- Additional Information (Clock 11) was received on 20 July 2022

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Abbreviations

bp	base pair
bw	body weight
CP4 EPSPS5	enolpyruvylshikimate 3-phosphate synthase.
DMO	dicamba mono-oxygenase
dw	dry weight
ELISA	enzyme-linked immunosorbent assay
ERA	environmental risk assessment
F_TT	2,4-D and FOPs dioxygenase protein
FOPs	aryloxyphenoxypropionate (AOPP) acetyl coenzyme A carboxylase inhibitors
fw	fresh weight
GLP	good laboratory practice
GMO	genetically modified organism
HGT	horizontal gene transfer
HR	homologous recombination
JSA	junction sequence analysis
LB	left border
MS	mass spectrometry
NGS	next generation sequencing
Nos	nopaline synthase
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PAT	phosphinothricin-N-acetyltransferase
PCR	polymerase chain reaction
PMEM	post-market environmental monitoring
RB	right border
rbcS	Rubisco
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
T-DNA	transfer-deoxyribonucleic acid
UTR	untranslated region

Appendix A – List of relevant publications identified by the applicant through systematic literature searches (January 2009–May 2022)

Reference
Yang H, Qi Y, Goley ME, Huang J, Ivashuta S, Zhang Y, Sparks OC, Ma J, van Scoyoc BM, Caruano-Yzermans AL, King-Sitzes J, Li X, Pan A, Stoecker MA, Wiggins BE and Varagona MJ, 2018. Endogenous tassel-specific small RNAs mediated RNA interference enables a novel glyphosate-inducible male sterility system for commercial production of hybrid seed in *Zea mays* L. PLoS One, 1–17.

Appendix B – Additional studies

List of additional studies performed by or on behalf of the applicant regarding the evaluation of the safety of maize MON 87429 for humans, animals, or the environment.

Study identification	Title
M-475440-01-1	Blanck, M. 2014. PAT/pat protein acute toxicity by oral gavage in mice. Bayer CropScience, Study Number SA 13205
MSL0026454	Smedley, J.W. 2015. An Acute Toxicity Study of *E. coli* produced CP4 EPSPS Protein by Oral Gavage in Mice. Monsanto Technical Report MSL0026454. St. Louis, Missouri.

Appendix C – Statistical analysis and statistically significant findings in the 28-day toxicity study in mice on the microbially produced DMO and FT_T proteins and 90-day toxicity study in rats on maize MON 87429

C.1 Statistical analysis in the 28-day toxicity study in mice on *E. coli*-produced DMO 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 four test diet groups (high, medium-high, medium and low MON 87429 DMO protein groups) separately with the vehicle group. The analysis was performed for male and female mice separately at 5% level of significance. Continuous endpoints were analysed with a linear mixed model (fixed effect: diet; random effect: block; for locomotor activity data, additional fixed effects were time and the interaction diet-time); for end points 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: Statistically significant findings in 28-day study on *E. coli* produced DMO protein in mice

Statistically significant parameter/ endpoint	Finding	GMO Panel interpretation
Body weight and body weight gain	Reduced in 500 mg/kg bw per day males up to day 14.	Not seen at the top dose level or over the second half of the study. Not an adverse effect of treatment.
Food consumption.	Reduced versus vehicle controls but not BSA controls. In males of the 100, 500 and 1,000 mg/kg bw per day groups (up to 20%)	Associated with administration of a high level of protein. No consistent impact on body weight. Not an adverse effect of treatment.
Red cell distribution width	Increased (10%) in 10, 500 and 1,000 mg/kg bw per day females versus vehicle controls but not BSA controls.	Vehicle control value is unusually low (outside HCD range). Not an adverse effect of treatment.
Bilirubin levels	Increased (100%) in males of the top dose group.	Primarily due to a high value in one animal. No other indications of adverse effects on the liver. Not an adverse effect of treatment.
Albumin: Globulin ratio	Decreased (7%) in 500 mg/kg bw per day females versus vehicle control but not BSA controls.	Small magnitude. Not seen at the high dose. Not an adverse effect of treatment
Globulin levels	Increased (6%) in 1,000 mg/kg bw per day females versus vehicle control but not BSA controls.	Small magnitude, within normal variation. Not an adverse effect of treatment.
Urea nitrogen	Increased (15%) in 500 mg/kg bw per day females.	Not seen at the top dose level. Within normal variation (value is the same as HCD mean). Not an adverse effect of treatment
Epididymis weight (absolute and relative to body wt)	Increased (10%) in 10 mg/kg bw per day group.	Not seen at higher doses. Small magnitude, within normal variation. Not an adverse effect of treatment.
Testes weights (absolute and relative to body wt)	Increased (17%) in the 10 and 500 mg/kg bw per day groups	No dose–response and not seen at the top dose level. Small magnitude, within normal variation. Not an adverse effect of treatment.

C.2 Statistical analysis in the 28-day toxicity study in mice on *E. coli* produced FT_T protein in mice

The following endpoints were statistically analysed: body weights, body weight changes, food consumption, clinical pathology values (as applicable), absolute and relative organ weights, functional observational battery (FOB) data, locomotor activity and histopathological data. For all continuous endpoints, mean, standard deviation in terms of the standardised effect sizes (SES) of each dose group for each sex, variable and period or time interval were reported. The main statistical analysis compared each of the three test diet groups (high, medium and low FT_T protein groups) separately with the vehicle group.

The analysis was performed for male and female mice separately at 5% level of significance. Continuous endpoints were analysed with a linear mixed model (fixed effect: diet; random effect: block; for locomotor activity data, additional fixed effects were time and the interaction diet-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 control diet groups. Missing data were considered by the Panel and found not to have an impact on the results.

Table C.2: Statistically significant findings in 28-day study on *E. coli* produced FT_T protein in mice

Statistically significant parameter/ endpoint	Finding	GMO Panel interpretation
Body weight/body weight gain	Increased in mid-dose females at week 2, when compared to the vehicle control group.	Small magnitude. Not present at the top dose or at week 4. Within normal variation. Not an adverse effect of treatment.
Grooming	Increased in mid-dose females.	Evident pre-test, not present at the top dose, within normal variation. Not an adverse effect of treatment.
Hindlimb foot splay	Increased (30%) in mid-dose males.	Not present at the top dose, within normal variation. Not an adverse effect of treatment.
Body temperature	Increased (0.5°C) in mid-dose females, when compared to the vehicle control group only	Not present in high dose females, BSA control is 0.37°C above basal control. Not an adverse effect of treatment.
Haemoglobin	Decreased (4%) in low-dose females, when compared to the vehicle control group.	Small magnitude, within normal variation, no dose response. Not an adverse effect of treatment.
Lymphocyte and total WBC count	Decreased (30%–40%) in top dose females. WBC change is driven by lymphocytes.	Within normal variation, no associated pathology findings. Reduction in the number of mice with high lymphocyte counts, giving small spread within the test group. No individuals with very low counts. Not an adverse effect of treatment.
Neutrophils (%)	Increased in top dose females (40%). Absolute count unaffected.	Secondary to reduced lymphocyte/WBC count, within normal variation. Not an adverse effect of treatment.
Sorbitol dehydrogenase activity	Increased (50%) in mid- and top dose males when compared to the vehicle control group.	No increase versus BSA control, within normal variation. Two mice in the basal control group had atypically low values. No associated pathological findings. Not an adverse effect of treatment.
Total protein	Decreased (5%) in mid-dose males when compared to the vehicle control group.	Not present at the top dose, small magnitude, no change versus BSA control group, within normal variation. Not an adverse effect of treatment.

Statistically significant parameter/ endpoint	Finding	GMO Panel interpretation
Creatinine	Increased (500%) in top dose females when compared to the vehicle control group.	Within normal variation (same as HCD mean). The values are considered to be secondary to an increase in the ingested protein content as the BSA control is also increased markedly (350% of basal control). There were fewer histopathological findings in kidney of the top dose females than in the basal control females. Not an adverse effect of treatment.
BUN	Increased (25%) in BSA control and all treated female groups when compared to the vehicle control group.	Basal control is below the HCD range. All test group means within 3% of BSA mean and 4% of HCD mean. Within normal variation, no associated pathology findings. Not an adverse effect of treatment.
Spleen weight (absolute and relative to body weight)	Increased (15%–20%) in mid-dose females when compared to the vehicle control group.	No associated haematology or pathology changes. No dose response, within 5% of BSA control value and below HCD mean; basal control value below HCD range (relative). Within normal variation. Not an adverse effect of treatment.

C.3 Statistical analysis of the 90-day study on maize MON 84279 in rats

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

The main statistical analysis compared rats consuming high- and low-dose test diets with those consuming the control diet. The statistical analysis of continuous endpoints was performed using linear mixed models, applied separately for each parameter and period. For food consumption data (with cage-based observations), the model included treatment, sex and treatment-by-sex interaction as fixed effects; replicate-within-sex was the random effect. For body weight data, organ weights (absolute and relative), clinical pathology parameters and FOB evaluations (all with individual-level observations), the fixed effects were treatment, sex and treatment-by-sex interaction; the random effects were replicate-within-sex and the interaction of replicate and dose within sex (the latter representing the cage effect). For locomotor activity data, the model was expanded to include time interval as an additional fixed effect and terms for the interaction of time interval with all the other factors. For all the models, in case the sex-by-treatment interaction was significant (and in any case for sex-specific parameters) a sex-specific analysis was performed. For categorical parameters (microscopic findings) the high- and low-dose groups were compared with the control group for each sex using Fisher's exact test.

Historical control data were provided for body weight data, food consumption, clinical pathology parameters and organ weights (absolute and relative) and used to assess the statistical differences identified for such parameters in the study. Missing data were considered by the Panel and found not impacting the results.

Table C.3: Statistically significant findings in 90-day study on maize MON 84279 in rats

Statistically significant parameter/ endpoint	Finding	GMO Panel interpretation
Body weight	Increased (5%) in low dose males. Reduced (5%–10%) in top dose females on weeks 6, 8, 10.	Small magnitude. No significant effects on terminal body weights. Not an adverse effect of treatment.
Body weight gain	Increased (10%) in low dose males (weeks 0–6 and 0–8). Reduced (20%) in low dose females (weeks 0–6) and in top dose females at most timepoints.	Within normal variation. No significant effects on terminal body weights. Not an adverse effect of treatment.
Food consumption	Reduced (10%) in both sexes combined weeks 5–10.	Small magnitude. Within normal variation. No significant effects on terminal body weights. Not an adverse effect of treatment.
Basophil count	Reduced (50%) in low dose males.	Not seen at the high dose. Within normal variation. Not an adverse effect of treatment.
Mean corpuscular haemoglobin concentration.	Reduced (2%) in both top dose groups combined.	Small magnitude. No effect on total haemoglobin. Within normal variation. Not an adverse effect of treatment.
Cholesterol	Increased (20%) in low and top dose males	Small magnitude. Within normal variation. Not an adverse effect of treatment.
Total protein	Increased (6%) in top dose males	Small magnitude. Not an adverse effect of treatment.
Globulin	increased (7%) in top dose males	Small magnitude. Not an adverse effect of treatment.
High density lipoprotein Low density lipoprotein	Increased (15%–25%) in top dose males; HDL decreased (10%) in top dose females	Small magnitude. Not consistent across sexes. Within normal variation. Not an adverse effect of treatment.
Albumin	Decreased (4%) in top dose females	Small magnitude. Within normal variation. Not an adverse effect of treatment.
Ovary/oviduct weights relative to body weight	Increased (10%) in the top dose group	Small magnitude. Within normal variation. No histopathology changes. Not an adverse effect of treatment.
Brain weight relative to body weight.	Increased (5%) in the top dose group	Small magnitude. Within normal variation. No histopathology changes or clinical signs. Not an adverse effect of treatment.

Appendix D – Animal dietary exposure

Table D.1: Animal dietary exposure to DMO, PAT, FT_T and CP4 EPSPS proteins (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%	Dietary exposure (μg/kg bw per day)			
			DMO	PAT	FT_T	CP4 EPSPS
Broiler 0.12/1.7	Grain	70	118.6	42.5	2,520	33.1
	Gluten feed	10	44	15.9	936	12.3
	Gluten meal	10	120.3	43.1	2,556	33.6
	Total	90	283	101	6,012	79
Finishing pig 100/3	Grain	70	50.4	18.1	1,071	14.1
	Gluten feed	20	37.4	13.44	795.6	10.4
	Gluten meal	10	51.1	18.3	1,086	14.2
	Total	100	139	50	2,953	39
Lactating dairy cow 650/25	Gluten feed	20[A]	48	17.2	1,020	13.4
	Gluten meal	20	131.1	46.9	2,785	36.6
	Silage	60	484.6	30	2,307	189.2
	Total	100	664	94	6,113	239

[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.