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Assessment of genetically modified maize MON 87419 for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2017-140)

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Abstract

Genetically modified maize MON 87419 was developed to confer tolerance to dicamba- and glufosinate-based herbicides. These properties were achieved by introducing the *dmo* and *pat* expression cassettes. The molecular characterisation data and bioinformatic analyses do not identify issues requiring food/feed safety assessment. None of the identified differences in the agronomic/phenotypic and compositional characteristics tested between maize MON 87419 and its conventional counterpart needed further assessment, except for the levels of arginine and protein in grains which did not raise safety and nutritional concerns. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the dicamba mono-oxygenase (DMO) and phosphinothricin *N*-acetyltransferase (PAT) proteins as expressed in maize MON 87419. The GMO Panel finds no evidence that the genetic modification impacts the overall safety of maize MON 87419. In the context of this application, the consumption of food and feed from maize MON 87419 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that maize MON 87419 is as safe as the conventional counterpart and non-GM maize varieties tested, and no post-market monitoring of food/feed is considered necessary. In the case of accidental release of viable maize MON 87419 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 87419. The GMO Panel concludes that maize MON 87419 is as safe as its conventional counterpart and the tested non-GM maize varieties with respect to potential effects on human and animal health and the environment.

Keywords: GM, genetic engineering, maize (*Zea mays*), MON 87419, DMO, PAT, import and processing

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Summary

Following the submission of application EFSA-GMO-NL-2017-140 under Regulation (EC) No 1829/2003 from Monsanto Europe S.A. (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 (*Zea mays* L.) MON 87419 according to Regulation (EU) No 503/2013). The scope of application EFSA-GMO-NL-2017-140 is for import, processing, and food and feed uses within the European Union (EU) of maize MON 87419 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 87419 according to the scope of the application EFSA-GMO-NL-2017-140. The GMO Panel conducted the assessment of maize MON 87419 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 87419 contains a single insert consisting of one copy of the *dmo* and *pat* expression cassettes. Updated bioinformatics analyses of the sequences encoding the newly expressed proteins and open reading frames (ORFs) present within the insert or spanning the junctions between the insert and genomic DNA, do not raise any safety concerns. The stability of the inserted DNA and of the introduced trait is confirmed over several generations. The methodology used to quantify the levels of the dicamba mono-oxygenase (DMO; consisting of two variants, DMO + 7 and DMO + 12) and PAT proteins is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of plant- and microbe-produced DMO and PAT proteins, indicate that these proteins are equivalent and the microbial-derived proteins can be used in the safety studies.

Considering the selection of test materials, the field trial sites and the associated management practices, 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 87419 and its conventional counterpart needed further assessment, except for the levels of arginine and protein in grains which did not raise safety and nutritional concerns. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the DMO and PAT proteins as expressed in maize MON 87419 and finds no evidence that the genetic modification would change the overall allergenicity of maize MON 87419. In the context of this application, the consumption of food and feed from maize MON 87419 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that maize MON 87419 is as safe as the conventional counterpart and non-GM maize varieties tested, and no post-market monitoring of food/feed is considered necessary.

Considering the introduced traits, the outcome of the agronomic and phenotypic analysis and the routes and levels of exposure, maize MON 87419 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 87419.

The GMO Panel considered the overall quality of the performed literature searches acceptable. Based on the relevant publications identified through the literature searches, the GMO Panel does not identify any safety issue pertaining to the intended uses of maize MON 87419.

The GMO Panel concludes that maize MON 87419 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-2017-140 is for food and feed uses, import and processing of maize MON 87419 and does not include cultivation in the European Union (EU). Maize MON 87419 was developed to confer tolerance to dicamba- and glufosinate-based herbicides.

1.1. Background

On 5 April 2017, the European Food Safety Authority (EFSA) received from the Competent Authority of The Netherlands application EFSA-GMO-NL-2017-140 for authorisation of MON 87419 (Unique Identifier MON-87419-8), submitted by Monsanto Europe S.A. (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003[1]. Following receipt of application EFSA-GMO-NL-2017-140, EFSA informed EU Member States (MS) and the European Commission (EC), and made the application available to them. Simultaneously, EFSA published summary of the application.[2]

EFSA checked the application for compliance with the relevant requirements of Regulation (EC) No 1829/2003 and Regulation (EU) No 503/2013[3], with the EFSA guidance documents, and, when needed, asked the applicant to supplement the initial application. On 17 July 2017, EFSA declared the application valid.

From validity date, EFSA and the Panel on Genetically Modified Organisms of the European Food Safety Authority (referred to hereafter as 'GMO Panel') endeavoured to respect a time limit of 6 months to issue a scientific opinion on application EFSA-GMO-NL-2017-140. Such time limit was extended whenever EFSA and/or GMO Panel requested supplementary information to the applicant. According to Regulation (EC) No 1829/2003, any supplementary information provided by the applicant during the risk assessment was made available to the EU Member States and European Commission (for further details, see the section 'Documentation', below).

In accordance with Regulation (EC) No 1829/2003, EFSA consulted the nominated risk assessment bodies of EU Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC[4]. The EU Member States had 3 months to make their opinion known on application EFSA-GMO-NL-2017-140 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 87419 in the context of its scope as defined in application EFSA-GMO-NL-2017-140.

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 assessment of maize MON 87419 on the valid application EFSA-GMO-NL-2017-140, additional information provided by the applicant during the risk assessment, relevant scientific comments submitted by the Member States and relevant peer-reviewed scientific publications. As part of this comprehensive information package, the GMO Panel received additional

[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-2017-00263.
[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-2017-00263

unpublished studies submitted by the applicant in order to comply with the specific provisions of Regulation (EU) No 503/2013. A list of these additional unpublished studies is provided in Appendix A.

2.2. Methodologies

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

For this application, in the context of the contracts OC/EFSA/GMO/2021/06, OC/EFSA/GMO/2018/02, and EOI/EFSA/SCIENCE/2020/01 – CT02GMO, the contractors performed preparatory work for the evaluation of the applicant's methods applied for the bioinformatics, statistical analysis and statistical analysis of the 90-day toxicity study on maize MON 87419.

3. Assessment

3.1. Introduction

Maize MON 87419 was developed to confer tolerance to dicamba- and glufosinate-based herbicides. Maize MON 87419 expresses two variants of the DMO protein, DMO + 7 and DMO + 12, and PAT protein. The DMO protein demethylates dicamba, producing 3,6-dichlorosalicylic acid and formaldehyde, conferring tolerance to dicamba-based herbicides. The PAT protein is a phosphinothricin acetyltransferase enzyme that confers tolerance to the glufosinate ammonium containing herbicides.

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

3.2. Systematic literature review[7]

The GMO Panel assessed the applicant's literature searches on maize MON 87419, which include a scoping review, according to the guidelines given in EFSA (2010, 2019b).

A systematic review as referred to in Regulation (EU) No 503/2013 has not been provided in support to the risk assessment of application EFSA-GMO-NL-2017-140. 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 87419 at present. The GMO Panel considered the overall quality of the performed literature searches acceptable.

The literature searches identified five records on maize MON 87419. The relevant records are listed in Appendix B.

None of the relevant records identified through the literature searches reported information pointing to safety issues associated with maize MON 87419 relevant to the scope of this application.

3.3. Molecular characterisation[8]

3.3.1. Transformation process and vector constructs

Maize MON 87419 was developed by *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*)-mediated transformation of embryogenic callus derived from post-pollinated maize ear of LH244, with plasmid vector PV-ZMHT507801. The PV-ZMHT507801 vector contained two T-DNAs, T-DNA I with the *dmo* and *pat* expression cassettes between the right and left borders which confer herbicide resistance, and T-DNA II with the *cp4 epsps* expression cassette between the right and left borders, which was used for the selection of transformed plants. Although both T-DNAs were initially inserted during transformation, they were not genetically linked and T-DNA II was eliminated by crossing, therefore only T-DNA I is present in the maize MON 87419, as described below.

T-DNA I carries two expression cassettes containing the following genetic elements:

- The *dmo* expression cassette contains the following genetic elements: the promoter of peanut chlorotic streak caulimovirus, the intron and flanking untranslated sequence of the rice (*Oryza sativa*) actin 1 gene, the targeting and leader sequence of the *shkG* gene from *Petunia hybrida*,

[7] Dossier: Part II – Section 7; additional information provided: 29/11/2018, 09/03/2020, 23/09/2022.
[8] Dossier: Part II – Section 1.2; additional information: 20/12/2017, 26/11/2018, 09/03/2020, 05/05/2020 and 23/09/2022.

the codon-optimised coding sequence for the dicamba mono-oxygenase (*dmo*) protein of *Stenotrophomonas maltophilia*, and the 3′ untranslated region from the heat shock protein from wheat (*Triticum aestivum*).

– The *pat* expression cassette contains the following genetic elements: the promoter, leader sequence and intron of the ubiquitin gene from big bluestem grass (*Andropogon gerardii*); the codon-optimised coding sequence of the phosphinothricin *N*-acetyltransferase gene (*pat*) from *Streptomyces viridochromogenes*; and the 3′ untranslated region of the RA5B precursor gene from rice (*Oryza sativa*), encoding an alpha-amylase/trypsin inhibitor that directs polyadenylation of mRNA.

T-DNA II carries one expression cassette containing the following genetic elements:

The *cp4 epsps* expression cassette consisting of the promoter, 5′ UTR and intron sequence of the *act1* (*Ract1*) gene from *Oryza sativa*, the chloroplast transit peptide (TS-CTP2) of the *shkG* gene from *Arabidopsis thaliana*, the coding sequence of the *aroA* gene from *Agrobacterium* sp. encoding the CP4 EPSPS protein and the 3′ untranslated sequence of the *nopaline synthase* (*nos*) gene from *Agrobacterium tumefaciens*.

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

3.3.2. Transgene constructs in the GM plant

Molecular characterisation of maize MON 87419 was performed by next generation sequencing (NGS) and junction sequence analysis (JSA) in order to determine insert copy number and to confirm the absence of plasmid backbone and T-DNA II sequences, and NGS sequencing on PCR amplified fragments to determine size and organisation of the inserted sequences. The approach used was acceptable both in terms of coverage and sensitivity.

NGS/JSA of the whole genome indicated that maize MON 87419 contains a single insert, consisting of a single copy of the T-DNA I in the same configuration as in the PV-ZMHT507801 transformation vector. NGS/JSA also confirmed the absence of vector backbone and T-DNA II sequences in the maize genome.

The nucleotide sequence of the entire insert of maize MON 87419 together with 1,246 bp of the 5′ and 1,251 bp of the 3′ flanking regions were determined. The results were in line with those shown by the NGS/JSA analyses. The insert of 6,762 bp is identical to the T-DNA I of PV-ZMHT507801, with the exception of 215 bp deleted from the T-DNA RB and 231 bp deleted from T-DNA LB. A comparison of the flanking regions with the pre-insertion locus indicated a 602-bp deletion at the insertion site.

The possible interruption of known endogenous maize genes by the insertion in maize MON 87419 was evaluated by bioinformatics analyses of the pre-insertion locus and of the genomic sequences flanking the insert. The results of these analyses do not indicate the interruption of any known endogenous gene in maize MON 87419.

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

Two variants of the DMO protein are present in maize MON 87419, DMO + 7 and DMO + 12. Updated bioinformatics analyses of the amino acid sequence of the newly expressed DMO + 12 (which contains the DMO + 7) amino acid sequence and PAT proteins reveal no significant similarities to toxins and allergens. In addition, updated bioinformatic analyses of the newly created open reading frames (ORFs) within the insert and spanning the junctions between the insert and genomic DNA also do not indicate significant similarities to toxins and allergens.

In order to assess the possibility for horizontal gene transfer (HGT) by homologous recombination (HR), the applicant performed a sequence identity analysis for maize MON 87419 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 87419 expresses two new proteins: DMO (consisting of two variants, DMO + 7 and DMO + 12) and PAT. Given the technical restraints in producing large enough quantities from plants, these proteins were recombinantly produced in *Escherichia coli*. A set of biochemical methods was employed to demonstrate the equivalence between the maize- and *E. coli*-derived DMO and PAT

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

The DMO precursor protein undergoes alternative processing, resulting in two monomeric forms (DMO + 7 and DMO + 12), referred to hereafter as DMO protein.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and western blot analysis showed that both plant- and microbe-produced DMO proteins had the expected molecular weight of ~39.5 kDa and were comparably immunoreactive to DMO protein-specific polyclonal antibodies. Glycosylation analysis demonstrated that none of the DMO proteins were glycosylated. Amino acid sequence analysis by mass spectrometry showed that both proteins matched the deduced sequence as defined by the inserted *DMO* gene. In addition, N-terminal sequencing analysis identified two forms of DMO present in maize MON 87419; one containing 7 (DMO + 7) and one containing 12 (DMO + 12) additional residues derived from the cleavage of the chloroplast transit peptide CTP4, included to target the DMO protein to the chloroplast. Due to the difference of only five residues, the two forms were indistinguishable by SDS–PAGE or western blot analysis and could not be physically separated during protein purification. They were therefore considered as a single DMO protein form. These data were consistent with the microbe-derived DMO protein which was designed to also contain the same 12 residues of CTP4 at its N-terminus. Functional equivalence was demonstrated by a biochemical *in vitro* activity assay which showed that maize and *E. coli*-derived proteins had comparable activity for the intended herbicide.

3.3.3.2. PAT protein characterisation and equivalence

SDS–PAGE and western blot analysis showed that both plant- and microbe-produced PAT proteins had the expected molecular weight of ~ 25 kDa and were comparably immunoreactive to PAT protein-specific polyclonal antibodies. Glycosylation detection analysis demonstrated that none of the PAT proteins were glycosylated. Amino acid sequence analysis by mass spectrometry and N-terminal sequencing methods showed that both proteins matched the deduced sequence as defined by the inserted *pat* gene. These data also showed that the plant-derived protein had its N-terminal methionine truncated. Such modifications are common in eukaryotic proteins (e.g. Polevoda and Sherman, 2000). Functional equivalence was demonstrated by a biochemical *in vitro* activity assay which showed that both proteins had comparable activity for the intended herbicide.

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

3.3.4. Information on the expression of the insert

Protein levels of DMO (consisting of DMO + 7 and DMO + 12), and PAT were analysed by an enzyme-linked immunosorbent assay (ELISA) in material harvested from a field trial across five locations in the US during the 2013 growing season. Samples analysed included leaf (V3), root (V3), forage (R5) and grains (R6) from plants treated with the combination of dicamba and glufosinate, and forage (R5) and grains (R6) from plants not treated with herbicides. The mean values, standard deviations and ranges of protein expression levels in grains (n = 20) and forage (n = 20) of the DMO and PAT proteins used to estimate human and animal dietary exposure (see Section 3.5.5.2) are reported in Table 1.

Table 1: Mean values, standard deviation and ranges of newly expressed proteins in grains [µg/g dry weight (dw) and µg/g fresh weight (fw)] and forage (µg/g dw) from maize MON 87419 (n = 20)[a]

Tissue	Dicamba and glufosinate treatment			
	Not treated		Treated	
	µg/g dry weight (dw)	µg/g fresh weight (fw)	µg/g dry weight (dw)	µg/g fresh weight (fw)
Grains (R6)				
DMO	0.20[b] ± 0.034[c] (0.14–0.26)[d]	0.18 ± 0.030 (0.13–0.23)	0.19 ± 0.048 (0.14–0.31)	0.17 ± 0.044 (0.13–0.29)
PAT	1.4 ± 0.30 (0.88–2.1)	1.3 ± 0.26 (0.78–1.9)	0.93 ± 0.27 (0.56–1.6)	0.85 ± 0.25 (0.50–1.4)
Forage (R5)				
DMO	8.0 ± 2.8 (4.6–16)		6.0 ± 2.7 (3.1–14)	
PAT	5.6 ± 2.2 (2.9–11)		5.0 ± 1.6 (2.8–8.5)	

(a): n = 11 and n = 17 for DMO in grains treated and not treated with the intended herbicides, respectively.
(b): Mean value.
(c): Standard deviation.
(d): Range.

3.3.5. Inheritance and stability of inserted DNA

Genetic stability of maize MON 87419 insert was assessed by NGS/JSA from five generations (R3, R4, R5, R3F1 and R4F1), while segregation analysis was performed by PCR-based analysis from three generations (BC1F1, BC2F1 and BC2F2). The results indicate that all the plants tested retained the single copy of the insert and flanking regions, which were stably inherited in subsequent generations. The results support the presence of a single insertion, segregating in a Mendelian fashion.

3.3.6. Conclusion on molecular characterisation

The molecular characterisation data establish that maize MON 87419 contains a single insert consisting of one copy of the *dmo* and the *pat* expression cassettes. Bioinformatics analyses of the sequences encoding the newly expressed proteins and other ORFs within the insert or spanning the junctions between the insert and genomic DNA do not raise any safety concerns. The stability of the inserted DNA and of the introduced trait is confirmed over several generations. The methodology used to quantify the levels of the DMO (consisting of DMO + 7 and DMO + 12), and PAT proteins is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of plant- and microbe-produced DMO and PAT proteins, indicate that these proteins are equivalent and the microbial-derived proteins can be used in the safety studies.

3.4. Comparative analysis[9]

3.4.1. Overview of studies conducted for the comparative analysis

Application EFSA-GMO-NL-2017-140 presents data on agronomic and phenotypic characteristics, as well as on forage and grain composition of maize MON 87419 (Table 2). In addition, the application contains data on characteristics of grain and pollen from maize MON 87419 (see Appendix A).

[9] Dossier: Part II – Section 1.3 and 7; Additional information: 28/08/2017, 25/10/2017 and 20/12/2017.

Table 2: Main comparative analysis studies to characterise maize MON 87419 provided in the application EFSA-GMO-NL-2017-140

Study focus	Study details	Comparator	Non-GM reference varieties
Agronomic and phenotypic analysis	Field study, USA, 2013, 2014, 2016, 10 field trial sites[(a)]	NL6169	20[(b)]
Compositional analysis	Field study, USA, 2013, eight field trial sites[(a)]		15[(b)]

GM: genetically modified.
(a): The field trials were established in 2013 at Jackson, AR; Warren, IL; Clinton, IN; Pawnee, KA; York NE and Lehigh, PA. Four additional field trials used only for agronomic and phenotypic analysis were established in 2013 at Jefferson, IA and Miami, OH; in 2014 at York NE and in 2016 at Perquimans, NC. Two additional field trials used only for compositional analysis were established in 2013 at Boone, IA and Butler, MO. The field trials in York, NE, in 2013 and 2014 are considered as two distinct field trial sites (combinations location/year).
(b): Thirteen reference varieties were used for both the agronomic and phenotypic and the compositional analysis: Dekalb DKC57 73, Dekalb DKC59 34, Dekalb DKC63 43, Gateway 6116, H 9180, Lewis 7007, Mycogen 2M746, NC+ 5220, Phillips 713, Phillips 717, Stewart S588, Stewart S602 and Stine 9724. Seven reference varieties were used only for the agronomic and phenotypic analysis: Channel 213 88, LG2597, Mycogen J790, NH6280, Specialty 3656, Stewart S480 and Stine 9628. Two reference varieties were used only for the compositional analysis: Gateway 4148 and Midland Philips 7B15P.

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 87419 not exposed to the intended herbicides, maize MON 87419 exposed to the intended herbicides, the comparator maize NL6169 and four 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 87419, the application of a difference test (between the GM maize and the comparator) and an equivalence test (between the GM maize and the set of non-GM commercial reference varieties). The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).[10]

3.4.3. Suitability of selected test materials

3.4.3.1. Selection of the starting materials for the comparative analysis

Maize event MON 87419 was obtained using the non-GM maize inbred line LH244 as recipient inbred line. For the field trial studies, the transformed inbred line LH244 was crossed with the non-GM inbred line HCL645 to produce the GM hybrid used in the comparative analysis.

The comparator used in the field trials is the non-GM maize hybrid NL6169,[11] which has a similar genetic background as maize MON 87419 (as documented by the pedigree), and is therefore considered to be the conventional counterpart.

Both maize MON 87419 and its conventional counterpart have a comparative relative maturity (CRM) of 112, which is considered appropriate for growing in environments across North America, where the comparative field trials were conducted.

Commercial non-GM reference varieties with a CRM ranging from 107 to 115 were selected by the applicant and, at each selected site, four reference varieties were tested (see Table 2). On the basis of the provided information on the relative maturity classes, the GMO Panel considers that the selected non-GM maize reference varieties were appropriate for the comparative analysis.

3.4.3.2. Seed production and quality

Seeds of maize MON 87419 and its conventional counterpart used in the different field trials (see Table 2) were produced, harvested and stored under similar conditions. The genetic purity of maize MON 87419 seed lots was confirmed via event-specific PCR analysis. The mean germination rates of maize MON 87419 maize and control maize were between 98% and 99%. The GMO Panel considers

[10] In detail, the four outcomes are: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence).
[11] Non-GM hybrid line NL6129 corresponds to the cross between maize inbred lines LH244 and HCL645.

that the starting seed used as test material in the agronomic, phenotypic and compositional studies was of acceptable quality.

3.4.3.3. Conclusion on suitability

The GMO Panel is of the opinion that the maize MON 87419, its conventional counterpart and the non-GM maize reference varieties were properly selected, and are of adequate quality. Therefore, the test materials are considered suitable for the comparative analysis.

3.4.4. Representativeness of the receiving environments

3.4.4.1. Selection of field trial sites

The selected field trial sites were located in commercial maize-growing regions of the United States of America. The soil types of the selected fields were diverse,[12] corresponding to optimal, near-optimal and sub-optimal 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 monthly basis. No exceptional weather conditions were reported at any of the selected sites; therefore, the GMO Panel considers that the meteorological dataset falls within the historical range of climatic conditions normally occurring at these sites.

3.4.4.3. Management practices

The field trials included plots containing maize MON 87419, plots with the conventional counterpart and plots with non-GM maize reference varieties, managed according to local agricultural practices. In addition, the field trials included plots containing maize MON 87419 managed following the same agricultural practices, plus exposed to the intended dicamba- and glufosinate ammonium- containing herbicides. Dicamba-containing herbicide was applied at the V2–V4 growth stage and the glufosinate ammonium-containing herbicide at V4–V7 growth stage. The GMO Panel considers that the management practices, including sowing, harvesting and application of plant protection products were appropriate for the selected receiving environments.

3.4.4.4. Conclusion on representativeness

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

3.4.5. Agronomic and phenotypic analysis

3.4.5.1. Agronomic and phenotypic endpoints tested under field conditions

Thirteen agronomic and phenotypic endpoints[13] plus information on abiotic stressors, disease incidence and arthropod damage were collected from the field trials (see Table 2). The endpoints dropped ears and root lodged plants were not subjected to a formal statistical analysis because of lack of variability in the data.

The statistical analysis (Section 12) was applied to 11 endpoints, with the following results:

- For maize MON 87419 (not treated with the intended herbicides), a statistically significant difference compared with the conventional counterpart was identified for the endpoint final stand count, which fell under equivalence category I.
- For maize MON 87419 (treated with the intended herbicides), a statistically significant difference compared with the conventional counterpart was identified for the endpoint plant height, which fell under equivalence category I.

[12] Soil types of the field trials were silty clay loam, loam, silt loam and sandy loam.
[13] Early stand count, days to 50% pollen shed, days to 50% silking, stay green rating, ear height, plant height, dropped ears, stalk lodged plants, root lodged plants, final stand count, grain moisture, test weight and yield.

3.4.6. Compositional analysis

Maize MON 87419 grain and forage harvested from the field trials in the US in 2013 (Table 2) were analysed for 78 constituents (9 in forage and 69 in grains), including those recommended by OECD (2002). The statistical analysis was not applied to 13 grain constituents[14] because their concentrations in more than half of the samples were below the limit of quantification.

The statistical analysis was applied to a total of 65 constituents (9 in forage[15] and 56 in grain[16]); a summary of the outcome of the test of difference and the test of equivalence is presented in Table 3:

1) For maize MON 87419 not treated with the intended herbicides, statistically significant differences with the conventional counterpart were identified for 18 endpoints (16 in grain and 2 in forage). All these endpoints fell under equivalence category I or II except for protein levels in grain, which fell under equivalence category III (Table 3).
2) For maize MON 87419 treated with the intended herbicides, statistically significant differences with the conventional counterpart were identified for 18 endpoints (15 in grain and 3 in forage). All these endpoints fell under equivalence category I or II except for arginine expressed as % AA in grain, which fell under equivalence category IV (Table 3).

Table 3: Outcome of the comparative compositional analysis in grain and forage for maize MON 87419. The table shows the number of endpoints in each category

		Test of difference[(a)]			
		Treated[(c)]		Not-treated[(c)]	
		Not different	Significantly different	Not different	Significantly different
Test of equivalence[(b)]	Category I/II	44	17[(d)]	44	17[(d)]
	Category III/IV	2[(e)]	1[(f)]	2[(e)]	1[(f)]
	Not categorised	1[(g)]	–	1[(g)]	–
	Total endpoints	65		65	

(a): Comparison between maize MON 87419 and its conventional counterpart.
(b): Four different outcomes: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence). Not categorised means that the test of equivalence was not applied because of the lack of variation among the non-GM reference varieties.
(c): Treated/not treated with the intended herbicides dicamba and glufosinate-ammonium.
(d): Endpoints with significant differences between maize MON 87419 and the conventional counterpart and falling in equivalence category I-II. In forage, untreated only: none. Treated only: phosphorus. Both treated and untreated: acid detergent fibre (ADF), moisture. In grain, untreated only: leucine, valine, palmitic acid (C16:0), folic acid, α-tocopherol. Treated only: serine, stearic acid (C18:0), calcium, protein. Both treated and untreated: glycine, histidine, threonine, arachidic acid (C20:0), linoleic acid (C18:2), oleic acid (C18:1), manganese, zinc, carbohydrates, p-coumaric acid.
(e): Endpoints falling in equivalence category III–IV and with no significant differences between maize MON 87419 and the conventional counterpart. In forage, none. In grain, not treated only: arginine. Treated only: copper. Both treated and not treated: lysine.
(f): Endpoints with significant differences between maize MON 87419 and the conventional counterpart and falling in equivalence category III–IV. In forage, none. In grain, not treated only: protein. Treated only: arginine. Both treated and not treated: none. Quantitative results for these endpoints are reported in Table 4.
(g): Endpoints not categorised for equivalence and with no significant differences between maize MON 87419 and the conventional counterpart. In forage, none. In grain, not treated only: none. Treated only: none. Both treated and not treated: sodium.

[14] Furfural, capric acid (C10:0), caprylic acid (C8:0), lauric acid (C12:0), myristic acid (C14:0), myristoleic acid (C14:1), pentadecanoic acid (C15:0), pentadecenoic acid (C15:1), heptadecenoic acid (C17:1), γ-linolenic acid (C18:3), eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3), arachidonic acid (C20:4).
[15] Crude protein, crude fat, moisture, acid detergent fibre (ADF), neutral detergent fibre (NDF), ash, carbohydrates, calcium, phosphorus.
[16] Moisture, crude protein, crude fat, total dietary fibre (TDF), acid detergent fibre (ADF), neutral detergent fibre (NDF), ash, carbohydrates, alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, zinc, palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C20:1), behenic acid (C22:0), β-carotene, thiamine, riboflavin, niacin, pyridoxine, folic acid, α-tocopherol, *p*-coumaric acid, ferulic acid, phytic acid, raffinose.

The GMO Panel assessed all significant differences between maize MON 87419 and its conventional counterpart, taking into account potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties. Quantitative results for the endpoints showing significant differences between maize MON 87419 and its conventional counterpart and falling under category III/IV are given in Table 4.

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

	Endpoint	Maize MON 87419[(a)]		Conventional counterpart	Non-GM reference varieties	
		Not treated	Treated		Mean	Equivalence limits
Grain	Protein (% dw)	10.96*	10.81*	10.51	9.91	8.88–10.94
	Arginine (% AA)	3.98	3.97*	4.04	4.32	4.07–4.58

dw: dry weight; % AA: percentage total amino acids.
(a): Treated: treated with the intended herbicide dicamba and glufosinate-ammonium; not treated: treated only with conventional herbicides (see Section 3.4.4.3).
For the maize MON 87419, significantly different values are marked with an asterisk, while the outcomes of the test of equivalence are differentiated by greyscale backgrounds: white (equivalence category I or II), light grey (equivalence category III) and dark grey (equivalence category IV).

3.4.7. Conclusion on comparative analysis

Considering the selection of test materials, the field trial sites and the associated management practices, the GMO Panel concludes that the field trials were appropriate to support the comparative analysis.

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

- None of the differences identified in agronomic and phenotypic characteristics between maize MON 87419 and the conventional counterpart needs further assessment for potential environmental impact.
- None of the differences identified in forage and grain composition of maize MON 87419 and the conventional counterpart needs further assessment regarding food and feed safety except for the levels of arginine (treated) and protein (not treated) both in grains, which is further assessed in Section 3.5.

3.5. Food/feed safety assessment[17]

3.5.1. Effects of processing

Maize MON 87419 will undergo existing production processes used for conventional maize. No novel production process is envisaged. Based on the outcome of the comparative assessment, processing of the GM maize into food and feed products is not expected to result in products being different from those of conventional non-GM maize varieties.

3.5.2. Stability of newly expressed proteins

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

[17] Dossier: Part II – Section 1, 2, 3, 4; additional information: 20/12/2017; 10/09/2018; 26/11/2018; 29/11/2018; 01/08/2019; 14/02/2020; 18/03/2020; 03/06/2022; 23/09/2022.

3.5.2.1. Effect of temperature and pH on newly expressed proteins

The effects of temperature and pH on PAT and DMO proteins have been previously evaluated by the GMO Panel (EFSA GMO Panel, 2017b,c, 2021b, 2022a). The applicant provided additional studies on the PAT and DMO proteins as expressed in MON 87419 that were consistent with the outcome of previous studies on other PAT and DMO proteins (EFSA GMO Panel, 2013a). Briefly, samples of PAT and DMO proteins 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 PAT and DMO proteins have no or marginal activity after incubation at temperatures ≥ 55°C. In relation to the effect of pH on the PAT and DMO proteins, the molecular mass (~ 25 kDa for PAT and ~38 kDa for DMO) and immunoreactivity of the proteins were 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 PAT and DMO proteins have been previously evaluated by the GMO Panel (EFSA GMO Panel, 2017b,c, 2021b, 2022a). The applicant provided additional studies on the PAT and DMO proteins as expressed in MON 87419 that were consistent with the outcome of previous studies on other PAT and DMO proteins (EFSA GMO Panel, 2013a). Briefly, the resistance to degradation by pepsin of the PAT and DMO proteins were measured in separate studies in solutions containing pepsin and the test protein at pH 1.2. The integrity of the test proteins was analysed by gel electrophoresis followed by protein staining and Western blot analysis. No intact PAT and DMO proteins were detected within 30 s of incubation. In both cases, a short fragment of 3 kDa was present after 2 min of incubation but it was not observed after 5 min of incubation.

3.5.3. Toxicology

3.5.3.1. Testing of newly expressed proteins

Two proteins (PAT and DMO) are newly expressed in maize MON 87419. 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 87419.

Table 5: Intended effects of the NEPs in maize MON 87419

Protein	Intended effect in GM plant
PAT	The PAT protein confers tolerance to glufosinate-ammonium-based herbicides acting by acetylation of glufosinate-ammonium
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)

NEP previously assessed

The PAT protein was previously assessed by the GMO Panel in the context of other applications (e.g. EFSA, 2009a; EFSA GMO Panel, 2013a,b, 2017b,c, 2018, 2020) and no safety concerns for humans and animals were identified. This protein has been extensively characterised (Sections 3.3.3). Updated bioinformatics analyses revealed no similarities of the PAT protein with known toxins (Section 3.3.2). The GMO Panel is not aware of any new information that would change the previous conclusion on the safety of the PAT protein.

NEP never assessed before

A slightly different variant of the DMO protein was previously assessed by the GMO Panel in the context of soybean MON 87708, where it was present in two variants (i.e. DMO and DMO + 27), and no safety concerns for humans and animals were identified (e.g. EFSA GMO Panel, 2013a). In that context, upon request of the GMO Panel to confirm the safety, a 28-day toxicity study in mice with the mixture of the two variants was provided (EFSA GMO Panel, 2013a).

However, the GMO Panel noted that the DMO protein expressed in this GM maize MON87419 differs from the DMO protein in soybean MON 87708 for two amino acids in the internal amino acid

sequence, and for the presence of a different set of aa residues due to incomplete cleavage of the CTP, at the N terminus (i.e. DMO + 7 and DMO + 12 vs. DMO and DMO + 27).The GMO Panel assessed the safety profile of the DMO protein, taking into account molecular characterisation and bioinformatic analyses (Section 3.2), the history of safe use for consumption of the newly expressed protein, and in vitro (Section 3.5.2) and *in vivo* studies.

i) Molecular characterisation

The plant-produced MON 87419 DMO protein has been extensively characterised and its equivalence to the microbial-produced protein was demonstrated (Section 3.3.3).

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. maltophila* 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 87419 differ in their amino acid sequences from the wild-type DMO protein from *S. maltophilia*, both because of an extra amino-terminal amino acid remaining from the CTP4 sequence and an additional leucine at amino acid position two of the DMO protein itself.

iii c. Overall conclusion on the history of safe use

The source organism (*S. maltophilia*) is a 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 87419 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 proteins has been described in Section 3.4.2.

v) *In vivo* studies

The outcome of acute tox study and 28-day tox studies with the *E. coli*-produced MON 87419 DMO protein is described below.

Acute toxicity study[18]

An acute toxicity study in CD-1 mice administered the *E. coli*-produced MON 87419 DMO protein by gavage at the dose of 1,000 mg/kg bw showed no adverse effects.

28-Day repeated dose toxicity studies

Upon EFSA request, the applicant provided a 28-day toxicity study[19] in mice with an *E. coli*-produced DMO MON 87419 protein which was, however, not considered adequate by the GMO Panel, due to inappropriateness of the test item as a surrogate of the maize DMO MON 87419 protein. The *E. coli*-produced DMO protein was an 'iron-free' variant expressed as an insoluble fraction, and the functional equivalence with the maize MON 87419 DMO protein was not demonstrated. Furthermore, the description of the procedures applied to obtain the test item (including solubilisation and purification from inclusion bodies in presence of high concentrations of urea) led the GMO Panel to conclude that the test item was administered to mice in a denatured and inactive form.

The GMO Panel requested a new study with a protein structurally, biochemically and functionally equivalent to the maize DMO MON 87419 protein, taking into account that no technical limitations are envisaged in the purification of an intact and functional *E. coli*-produced DMO protein.

The applicant, instead of providing a 28-day study with a DMO MON 87419 protein, referred to the 28-day study with the DMO MON 87429 protein previously assessed by the GMO Panel in the frame of another application (EFSA GMO Panel, 2022a), supporting this choice with the comparison of the physico-chemical and functional properties of the MON 87419 DMO and MON 87429 DMO proteins.[20]

The EFSA GMO Panel confirmed the two proteins are equivalent, except for additional amino acid residues at the N-terminus.[21] Furthermore, updated bioinformatic analysis revealed no similarities of the additional amino acid sequence of the MON 87419 DMO protein with known toxins (see Section 3.3.2).

Based on the above information the GMO Panel agreed with the approach of referring to the 28-day study with the closely related DMO MON 87429 protein previously assessed (EFSA GMO Panel, 2022a), for which no adverse effects were observed up to the dose of 1,000 mg/kg bw per day in mice.

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 87419.

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 87419. Therefore, no further food and 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 and feed constituents have been identified in seed and forage maize MON 87419, except for arginine and protein in grains. These changes are considered not to represent a toxicological concern, considering the biological role of the affected constituents 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[22]

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 87419 related to the stability and expression of the insert, and to modifications of toxicological concern in the composition of maize MON 87419 (see Sections 3.3, 3.4 and 3.5.3). Therefore, animal studies with food/feed derived from maize MON 87419 are not considered necessary by the GMO

[18] Additional information 01/08/2019.
[19] Additional information 10/09/2018, 01/08/2019 and 18/03/2020.
[20] Additional information 03/06/2022.
[21] Amino acid residues at the N-terminus: DMO + 7 and DMO + 12 in MON 87419; DMO and DMO + 1 in MON 87429.
[22] Dossier: Part 1 – Section 1.4.4.1; additional information 14/02/2020.

Panel (EFSA GMO Panel, 2011a). In accordance with Regulation (EU) No 503/2013, the applicant provided a 90-day feeding study in rats fed with diets containing grains derived from maize MON 87419.

In this study, pair-housed Sprague Dawley rats (16 per sex per group; 2 rats per cage) were allocated to three groups using a randomised complete block design with 8 replications per sex.

Groups were fed diets containing maize MON 87419 grains from plants treated with the intended herbicide (dicamba and glufosinate) at 33% and 11% of inclusion level (the latter supplemented with 22% of the conventional counterpart) or the conventional counterpart ground grains (meal) at 33% of inclusion level.

The study was adapted from OECD test guideline 408 (1998), aligned with the guidance of the 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 (i.e. test item stability, homogeneity and concentration).

The stability of the test and control materials was not verified; however, in accordance with product expiration declared by the diet manufacturer, the constituents of the diets are considered stable for the duration of the treatment. The GMO Panel considered this justification acceptable. Diet preparation procedures and regular evaluations of the mixing methods guaranteed the homogeneity and the proper concentration of the test or control substances in them.

Event-specific PCR analysis confirmed the presence of the event MON 87419 in the GM grains and diets and excluded the presence of the event in the respective controls.

Both GM and control grains and diets were analysed for nutrients and potential contaminants (e.g. selected heavy metals, mycotoxins, pesticides). Balanced diets were based on the PMI Certified Rodent LabDiet 5002 prepared by TestDiet.

Feed and water were provided ad libitum. In-life procedures and observations and terminal procedures were conducted in accordance to OECD TG 408 (1998).

An appropriate range of statistical tests were performed on the results of the study. Detailed description of the methodology and of statistically significant findings identified in rats given diets containing grains/meal derived from maize MON 87419 is reported in Appendix C (Table C.1).

There were no test diet-related incidents of mortality or clinical signs. No test diet-related adverse findings were identified in any of the investigated parameters. A small number of statistically significant findings were noted but these were not considered adverse effects of treatment for one or more of the following reasons:

- were within the normal variation[23] for the parameter in rats of this age;
- were of small magnitude;
- were identified at only a small number of time intervals with no impact on the overall value;
- exhibited no consistent pattern with related parameters or endpoints;
- exhibited no consistency with increasing incorporation levels.

The GMO Panel concludes that this study is in line with the requirements of Regulation (EU) No 503/2013 and that no treatment related adverse effects were observed in rats after feeding diets containing maize MON 87419 grains at 33% or 11% for 90 days.

The study was conducted with the upper limit doses of 33%.[24] Since 2019, a 50% maize incorporation rate is used as the high dose (EFSA, 2014; Steinberg et al., 2019, 2020; EFSA GMO Panel, 2021b,c, 2022a,b,c). While the GMO Panel is reviewing the evidence regarding test diets

[23] Although animal used in a toxicology study are of the same strain, from the same supplier and are closely matched for age and body weight at the start of the study, they exhibit a degree of variability in the parameters investigated during the study. This variability is evident even within control groups. To help reach a conclusion on whether a statistically significant finding in a test group 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).

[24] 90-Day feeding study with maize MON 87419 was completed in 2015.

incorporating up to 50% and the potential to induce nutritional imbalance, currently the Panel considers that the upper limit of 33% is acceptable for this existing study.[25]

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 of the information obtained on the newly expressed protein, as no single piece of information or experimental method yielded sufficient evidence to predict allergenicity (Codex Alimentarius, 2009; EFSA GMO Panel, 2011a, 2017a; Regulation (EU)No 503/2013).

The *pat* and *dmo* genes originate from *S. hygroscopicus* and *S. maltophilia*, respectively, none of which are considered allergenic sources.

Updated bioinformatic analyses of the amino acid sequences of the PAT and DMO 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 and DMO proteins have been described in Section 3.5.2. Furthermore, the GMO Panel did not find an indication that the proteins PAT and DMO at the levels expressed in maize MON 87419 might be adjuvants.

Furthermore, the applicant provided information on the safety of the PAT and DMO proteins regarding their potential hazard to cause a celiac disease response. For such assessment, the applicant followed the principles described in the EFSA GMO Panel guidance document (EFSA GMO Panel, 2017a). The assessment of the DMO protein 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 position and nature of amino acids flanking the ELPA motif, such as the presence of two consecutive prolines and the charge and size of adjacent amino acids (EFSA GMO Panel, 2017a), the two 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 and/or DMO proteins in maize MON 87419 may be allergenic.

3.5.4.2. Assessment of allergenicity of the whole GM plant

The GMO Panel regularly reviews the available publications on food allergy to maize. However, maize is not considered a common allergenic food[26] (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 this GM maize MON 87419 with respect to that derived from the conventional counterpart and the non-GM reference varieties tested.

[25] Recent work (e.g. Steinberg, 2019; 2020) indicates that an acceptable upper limit for incorporation of maize into rodent diets is 50%. Many rodent studies evaluated by the GMO Panel were performed prior to 2019 and used upper incorporation rates of 33%. The GMO Panel considers that a 1.5-fold increase in incorporation rate is unlikely to identify any new hazards in the context of this application and therefore there is no reason to repeat these older studies using the new upper incorporation rate of 50%. This approach is consistent with Directive 2010/63/EU on the protection of animals used for scientific purposes.

[26] 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.

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 and PAT proteins newly expressed in MON 87419 maize. Dietary exposure was estimated based on protein expression levels reported in this application for the MON 87419 maize treated with the intended herbicides, the current available consumption data and feed practices, the foods and feeds currently available on the market and the described processing conditions.

For the purpose of estimating dietary exposure, the levels of newly expressed proteins in MON 87419 maize grains and forage were derived from replicated field trials (four replicates from five locations) in the 2013 US growing season (see Table 1, Section 3.3.4).

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). Since no specific consumption data were available on commodities containing, consisting of or obtained from MON 87419 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).[27] Corn oil, corn starch and corn syrup were excluded from the assessment since no proteins are expected to be present in these commodities.

For the acute dietary exposure estimations, the applicant directly assigned to processed commodities the mean value reported for the concentration of the newly expressed proteins in maize grains (0.17 μg/g fw for DMO and 0.85 μg/g fw for PAT). 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 from the EFSA consumption database were used.[28] Acute dietary exposure was estimated using for each population group the food commodity with the highest acute consumption among consumers only (95th or 97.5th percentile depending on the number of consumers),[29] and multiplying this value by the mean values of DMO and PAT proteins. The use of the highest acute consumption for only one food commodity could slightly underestimate the dietary exposure to DMO and PAT proteins in certain population groups. The highest acute dietary exposure was estimated in the age class 'Toddlers' with exposure estimates of 1.5 and 7.6 μg/kg bw day for DMO and PAT proteins, respectively. The most relevant food commodities in terms of contribution to the dietary exposure was sweet corn.

The GMO Panel estimated chronic dietary exposure to DMO and PAT proteins. Individual consumption data of the relevant food commodities were retrieved from the EFSA Consumption Database,[30] using dietary surveys with at least 2 days consumption and covering a total of 23 European countries.[31] Mean protein expression values on fresh weight basis are considered as the most adequate to estimate human dietary exposure (both acute and chronic) when working with raw primary commodities that are commonly consumed as processed blended commodities (EFSA, 2019a). Different recipes and factors were considered to estimate the amount of maize in the consumed commodities before assigning DMO and PAT proteins 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 3.8 mg/kg bw day and 0.8 mg/kg bw for DMO and PAT proteins, respectively. The main contributor to the exposure in the dietary survey with the highest estimates was sweet corn.

[27] https://www.efsa.europa.eu/en/food-consumption/comprehensive-database
[28] Data accessed: June 2015.
[29] EFSA (European Food Safety Authority), 2011. Evaluation of the FoodEx, the food classification system applied to the development of the EFSA Comprehensive European Food Consumption Database. EFSA Journal 2011;9(3):1970, 27 pp.
[30] Data accessed: April 2020.
[31] Austria, Belgium, Bulgaria, Cyprus, the Czech Republic, Germany, Denmark, Estonia, Finland, France, the United Kingdom, Greece, Croatia, Hungary, Ireland, Italy, Latvia, the Netherlands, Portugal, Spain, Romania, Slovenia and Sweden.
[32] 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 0.77 μg of PAT per gram of maize bread as compared to 0.85 μg/g in the maize grains.

Additional dietary exposure to the DMO and PAT proteins might occur via the consumption of pollen supplements under the assumption that these supplements contain pollen from MON 87419 maize. Consumption data on pollen supplements are available for few consumers across eight different European countries.[33] However, since no data on the presence of newly expressed proteins in pollen were available, the potential dietary exposure to DMO and PAT proteins from the consumption of pollen supplements could not be estimated.

3.5.5.2. Animal dietary exposure

Dietary exposure to PAT and DMO proteins in maize MON 87419 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 and gluten meal and maize forage).

A conservative scenario with 100% replacement of conventional maize products by the maize MON 87419 products was considered.

Mean levels (dry weight) of the newly expressed proteins in grains and forage from maize MON 87419 treated with the intended herbicides used for animal dietary exposure are listed in Table 1.

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/forage (OECD, 2002), and assuming that no protein is lost during their processing.

The applicant estimated dietary exposure to PAT and DMO proteins via the consumption of maize grains, gluten feed, gluten meal in broiler, finishing pig and lactating dairy cow, based on default values for animal body weight, daily feed intake and inclusion rates (percentage) of maize feedstuffs in diets and rations, as provided for the EU by OECD (2002). Estimated dietary exposure in the concerned animals is reported in Appendix D (Table D.1).

To further integrate the assessment, the GMO Panel estimated dietary exposure to PAT and DMO proteins across different livestock animal species (beef and dairy cattle, lamb, breeding swine and layer) following the consumption of forage, based on estimates for animal body weight, daily feed intake and inclusion rates of maize forage in animal diets as provided for the EU by OECD (2009). Estimated dietary exposure in the concerned animals is reported in Appendix D (Table D.2).

3.5.6. Nutritional assessment of GM food/feed

The intended trait of maize MON 87419 is herbicide tolerance, with no intention to alter nutritional parameters. However, levels of protein (not treated) and arginine (treated), both in grain, were significantly different from its conventional counterpart and showed a lack of equivalence with the set of non-GM reference varieties (Section 3.4.3). The biological relevance of protein and arginine, the role of maize as contributor to their total intake and the magnitude and direction of the observed changes were considered during the nutritional assessment.

3.5.6.1. Human nutrition

A relatively small increase of protein content (2.8%–4.2% as compared to its conventional counterpart) was observed in the GM-maize that does not imply any concern from nutritional point of view. Maize protein has a poor balance of essential amino acids, in particular due to the low levels of lysine and tryptophan. For arginine, which is not classified among the indispensable nine amino acids, a relative decrease of 1.7% was observed as compared to the conventional counterpart. Considering the magnitude of the change, and that intake of arginine is only needed under certain pathological conditions (EFSA NDA Panel, 2012), no nutritional concern is identified.

3.5.6.2. Animal nutrition

Maize grains are not considered a major source of protein in animals; therefore, a small increase of protein content in grains (2.8%–4.2% as compared to its conventional counterpart) rather improves the protein energy ratio.

Arginine is not considered essential amino acid, although Wu (2014) suggests that adequate supply of all amino acids is important to improve efficiency of animal production. The magnitude of the decrease of arginine content (% AA) observed in the GM maize grains as compared to the conventional counterpart (Table 4) does not represent a nutritional concern for animals.

[33] https://www.efsa.europa.eu/en/food-consumption/comprehensive-database. Data accessed: April 2020.

In animal nutrition, amino acids are also expressed as the percentage of dry weight (dw) to calculate directly the amount of each amino acid consumed daily by animals. According to Regulation (EC) No 767/2009[34], if, 'in complete or complementary feed, amino acids, vitamins and/or trace elements are indicated under the heading of analytical constituents, they shall be declared, along with the total amount thereof'. Therefore, variations in the levels of amino acids were also assessed taking in consideration data expressed as % dw (Table 6).

Table 6: Quantitative results (estimated means and equivalence limits) for compositional endpoints in grain expressed as percentage of dry weight

	Endpoint	Maize MON 87419[(a)]		Conventional counterpart	Non-GM reference varieties	
		Not treated	Treated		Mean	Equivalence limits
Grain	Protein (% dw)	10.96*	10.81*	10.51	9.91	8.88–10.94
	Arginine (% dw)	0.44*	0.43	0.43	0.42	0.39–0.46
	Alanine (% dw)	0.88*	0.86	0.84	0.77	0.66–0.87
	Glutamic acid (% dw)	2.29*	2.26	2.19	2.00	1.72–2.27
	Isoleucine (% dw)	0.40*	0.39	0.38	0.35	0.31–0.39
	Leucine (% dw)	1.52*	1.48	1.43	1.29	1.10–1.48
	Phenylalanine (% dw)	0.59*	0.57	0.56	0.51	0.44–0.58
	Proline	1.04*	1.02	0.99	0.92	0.82–1.01
	Serine (% dw)	0.56*	0.55	0.54	0.50	0.45–0.55
	Threonine (% dw)	0.40*	0.40	0.39	0.36	0.33–0.40
	Valine (% dw)	0.52*	0.51	0.50	0.47	0.42–0.51

% dw: percentage dry weight.
(a): Treated: treated with the intended herbicide dicamba and glufosinate-ammonium; not treated: treated only with conventional herbicides (see Section 3.4.4.3).
For the maize MON 87419, significantly different values are marked with an asterisk, while the outcomes of the test of equivalence are differentiated by greyscale backgrounds: white (equivalence category I or II), light grey (equivalence category III) and dark grey (equivalence category IV).

The magnitude of the increased arginine content with data expressed as % dw (equivalence category I or II) observed in the GM maize grains as compared to the conventional counterpart (Table 6), does not represent a nutritional concern for animals, and is likely associated to the higher protein content of GM maize grain. This result does not change the overall assessment described above for arginine expressed as % AA.

Furthermore, the magnitude of the increase of alanine, glutamic acid, isoleucine, leucine, phenylalanine, proline, serine, threonine and valine content (% dw) observed in GM maize grains as compared to the conventional counterpart (Table 6), is minimal and does not represent a nutritional concern for animals. Also in this case, the changes are likely associated to the higher protein content of GM maize grains.

3.5.7. Post-market monitoring of GM food/feed

The GMO Panel concluded that maize MON 87419, as described in this application, does not raise any nutritional concern and is as safe as the conventional counterpart and the non-GM reference 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.

[34] Regulation (EC) No 767/2009 of the European Parliament and of the Council of 13 July 2009 on the placing on the market and use of feed, amending European Parliament and Council Regulation (EC) No 1831/2003 and repealing Council Directive 79/373/EEC, Commission Directive 80/511/EEC, Council Directives 82/471/EEC, 83/228/EEC, 93/74/EEC, 93/113/EC and 96/25/EC and Commission Decision 2004/217/EC. OJ L 229, 1.9.2009, pp. 1–28.

3.5.8. Conclusions on the food/feed safety assessment

The proteins DMO and PAT newly expressed in maize MON 87419 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 87419. The GMO Panel found no evidence that the genetic modification impacts the overall safety of maize MON 87419. Based on the outcome of the comparative assessment and the nutritional assessment, the GMO Panel concludes that the consumption of maize MON 87419 does not represent any nutritional concern, in the context of the scope of this application. The GMO Panel concludes that maize MON 87419, 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-2017-140, which excludes cultivation, the environmental risk assessment (ERA) of maize MON 87419 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 87419 seeds/grains during transportation and/or processing (EFSA GMO Panel, 2010a).

3.6.1.1. Persistence and invasiveness of the GM plant

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

It is unlikely that the intended traits of maize MON 87419 will provide a selective advantage to maize plants, except when they are exposed to glufosinate-ammonium- and/or dicamba-containing herbicides. However, this fitness advantage will not allow the maize MON 87419 to overcome other biological and abiotic factors (described above) limiting plant's persistence and invasiveness. Therefore, the presence of the intended traits will not affect the persistence and invasiveness of the GM plant.

In conclusion, the GMO Panel considers that maize MON 87419 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 87419 grains.

3.6.1.2. Potential for gene transfer

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

Plant-to-microorganism gene transfer

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

[35] Dossier: Part II – Sections 5 and 6; additional information: 03/06/2022.

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, 2009b).

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, 2009b). 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 87419 revealed no sufficient sequence identity with known DNA sequences from bacteria which would facilitate homologous recombination. This result can be explained by the plant-codon optimisation applied to the genes of bacterial origin.

In summary, there is no indication for an increased likelihood of horizontal transfer of DNA from maize MON 87419 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 87419 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 3.6.1.1, 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-2017-140 (no cultivation) and the absence of target organisms into account, potential interactions of occasional feral maize MON 87419 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 87419 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 87419 with non-target organisms do not raise any environmental safety concern.

3.6.1.5. Interactions with abiotic environment and biogeochemical cycles

Given that environmental exposure to spilled grains or occasional feral maize MON 87419 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

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 87419, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for maize MON 87419 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 87419. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

3.6.2.1. Conclusion of the environmental risk assessment and monitoring plan

The GMO Panel concludes that it is unlikely that maize MON 87419 would differ from conventional maize varieties in its ability to persist under European environmental conditions. Considering the scope of application EFSA-GMO-NL-2017-140, interactions of occasional feral maize MON 87419 plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from maize MON 87419 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 87419 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 87419.

4. Overall conclusions

The GMO Panel was asked to carry out a scientific assessment of maize MON 87419 for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003. The molecular characterisation data establish that maize MON 87419 contains a single insert consisting of one copy of the *dmo* and *pat* expression cassettes. Updated bioinformatics analyses of the sequences encoding the newly expressed protein and other ORFs present within the insert or spanning the junctions between the insert and genomic DNA, do not raise any safety concerns. The 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 (consisting of DMO + 7 and DMO + 12), and PAT proteins is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of plant- and microbe-produced DMO and PAT 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 87419 and its conventional counterpart needed further assessment, except for the levels of arginine and protein in grains, which did not raise nutritional and safety concerns. The

GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the DMO and PAT proteins as expressed in maize MON 87419.

The GMO Panel finds no evidence that the genetic modification impacts the overall safety of maize MON 87419. In the context of this application, the consumption of food and feed from maize MON 87419 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that maize MON 87419 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 seeds from maize MON 87419 into the environment. The PMEM plan and reporting intervals are in line with the intended uses of maize MON 87419. Based on the relevant publications identified through the literature searches, the GMO Panel does not identify any safety issues pertaining to the uses of maize MON 87419. The GMO Panel concludes that maize MON 87419 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 (if appropriate)

- Letter from the Competent Authority of The Netherlands received on 5 April 2017 concerning a request for authorization of the placing on the market of genetically modified maize MON 87419 in accordance with Regulation (EC) No 1829/2003 by Monsanto Europe S.A./N.V. (EFSA Ref. EFSA-GMO-NL-2017-140; EFSA-Q-2017-00263)
- The application was made valid on 17 July 2017
- Additional information (1) was requested on 18 July 2017
- Additional information (1) was received on 28 August 2017
- Additional information (2) was requested on 27 September 2017
- Additional information (2) was received on 25 October 2017
- Additional information (3) was requested on 27 October 2017
- Additional information (3) was received on 20 December 2017
- Additional information (4) was requested by EURL on 30 October 2017
- Additional information (4) was received on 18 January 2018
- Additional information (5) was requested by EURL on 2 February 2018
- Additional information (5) was received on 1 March 2018
- Additional information (6) was requested on 28 March 2018
- Additional information (6) was received on 10 September 2018
- Additional information (7) was requested on 21 December 2018
- Additional information (7) was received on 1 August 2019
- Additional information (8) was requested on 19 July 2019
- Additional information (8) was received on 14 February 2020
- Additional information (9) was requested on 11 February 2020
- Additional information (9) was received on 18 March 2020
- Additional information (10) was requested on 27 May 2020
- Additional information (10) was received on 3 June 2022
- Additional information (11) was requested on 28 July 2022
- Additional information (11) was received on 23 September 2022
- Additional information provided on voluntary basis was submitted on 9 March 2020 and 5 May 2020

References

Berg G, Roskot N and Smalla K, 1999. Genotypic and phenotypic relationships between clinical and environmental isolates of Stenotrophomonas maltophilia. Journal of Clinical Microbiology, 37, 3594–3600.

Breiteneder H and Mills EN, 2005. Molecular properties of food allergens. Journal of Allergy and Clinical Immunology, 115, 14–23.

Calza L, Manfredi R and Chiodo F, 2003. Stenotrophomonas (Xanthomonas) maltophilia as an emerging opportunistic pathogen in association with HIV infection: A 10-year surveillance study. Infection, 31, 155–161. https://doi.org/10.1007/s15010-003-3113-6

Codex Alimentarius, 2009. Foods derived from modern biotechnology. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Rome, Italy. 85 pp. Available online. https://www.fao.org/docrep/011/a1554e/a1554e00.htm

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

D'Ordine RL, Rydel TJ, Storek MJ, Sturman EJ, Moshiri F, Bartlett RK, Brown GR, Eilers RJ, Dart C, Qi Y, Flasinski S and Franklin SJ, 2009. Dicamba monooxygenase: structural insights into a dynamic Rieske oxygenase that catalyzes an exocyclic monooxygenation. Journal of Molecular Biology, 392, 481–497.

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

EFSA (European Food Safety Authority), 2009a. Scientific opinion of the panel on genetically modified organisms on an application (Reference EFSA-GMO-CZ-2005-27) for the placing on the market of the insect resistant and herbicide-tolerant genetically modified maize MON 88017, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 2009;7(5):1075, 28 pp. https://doi.org/10.2903/j.efsa.2009.1075

EFSA (European Food Safety Authority), 2009b. Consolidated presentation of the joint Scientific Opinion of the GMO and BIOHAZ Panels on the 'Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants' and the Scientific Opinion of the GMO Panel on 'Consequences of the Opinion on the Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants on Previous EFSA Assessments of Individual GM Plants'. EFSA Journal 2009;7(6):1108, 107 pp. https://doi.org/10.2903/j.efsa.2009.1108

EFSA (European Food Safety Authority), 2010. Application of systematic review methodology to food and feed safety assessments to support decision making. EFSA Journal 2010;8(6):1637, 90 pp. https://doi.org/10.2903/j.efsa.2010.1637

EFSA (European Food Safety Authority), 2014. Explanatory statement for the applicability of the Guidance of the EFSA Scientific Committee on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed for GMO risk assessment. EFSA Journal 2014;12(10):3871, 25 pp. https://doi.org/10.2903/j.efsa.2014.3871

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

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

EFSA (European Food Safety Authority), Gomez Ruiz JA, Bresson J-L, Frenzel T and Paoletti C, 2019a. Statement on the human dietary exposure assessment to newly expressed proteins in GM foods. EFSA Journal 2019;17(7):5802, 18 pp. https://doi.org/10.2903/j.efsa.2019.5802

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

EFSA (European Food Safety Authority), Devos Y, Aiassa E, Muñoz-Guajardo I, Messéan A and Mullins E, 2022. Statement on the update of environmental risk assessment conclusions and risk management recommendations of EFSA (2016) on EU teosinte. EFSA Journal 2022;20(3):7228, 40 pp. https://doi.org/10.2903/j.efsa.2022.7228

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010a. Guidance on the environmental risk assessment of genetically modified plants. EFSA Journal 2010;8(11):1879, 111 pp. https://doi.org/10.2903/j.efsa.2010.1879

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010b. Statistical considerations for the safety evaluation of GMOs. EFSA Journal 2010;8(1):1250, 59 pp. https://doi.org/10.2903/j.efsa.2010.1250

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010c. Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal 2010;8(7):1700, 168 pp. https://doi.org/10.2903/j.efsa.2010.1700

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2011a. EFSA Panel on Genetically Modified Organisms (GMO); Scientific Opinion on guidance for risk assessment of food and feed from genetically modified plants. EFSA Journal 2011;9(5):2150, 37 pp. https://doi.org/10.2903/j.efsa.2011.2150

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2011b. Scientific Opinion on guidance on the Post-Market Environmental Monitoring (PMEM) of genetically modified plants. EFSA Journal 2011;9(8):2316, 40 pp. https://doi.org/10.2903/j.efsa.2011.2316

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2013a. Scientific Opinion on application EFSA-GMO-NL-2011-93 for the placing on the market of the herbicide-tolerant genetically modified soybean MON 87708 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 2013;11(10):3355, 30 pp. https://doi.org/10.2903/j.efsa.2013.3355

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). 2013b. Scientific opinion on applications EFSA-GMO-RX-T25 and EFSA-GMO-NL-2007-46 for the renewal of authorisation of maize T25, and for the placing on the market of herbicide-tolerant genetically modified maize T25, both for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience AG. EFSA Journal 2013;11(10):3356, 30 pp. https://doi.org/10.2903/j.efsa.2013.3356

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2015. Guidance on the agronomic and phenotypic characterisation of genetically modified plants. EFSA Journal 2015;13(6):4128, 44 pp. https://doi.org/10.2903/j.efsa.2015.4128

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Birch AN, Casacuberta J, De Schrijver A, Gralak MA, Guerche P, Jones H, Manachini B, Messean A, Nielsen EE, Nogue F, Robaglia C, Rostoks N, Sweet J, Tebbe C, Visioli F, Wal J-M, Eigenmann P, Epstein M, Hoffmann-Sommergruber K, Koning F, Lovik M, Mills C, Moreno FJ, van Loveren H, Selb R and Fernandez Dumont A, 2017a. Guidance on allergenicity assessment of genetically modified plants. EFSA Journal 2017;15(5):4862, 49 pp. https://doi.org/10.2903/j.efsa.2017.4862

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Birch AN, Casacuberta J, De Schrijver A, Gralak MA, Jones H, Manachini B, Mess ean A, Nielsen EE, Nogue F, Robaglia C, Rostoks N, Sweet J, Tebbe C, Visioli F, Wal J-M, Ardizzone M, Devos Y, Gomes A, Liu Y, Neri FM and Olaru I, 2017b. Scientific Opinion on an application by Dow AgroSciences LLC (EFSA-GMO-NL-2011-91) for the placing on the market of genetically modified herbicide-tolerant soybean DAS68416-4 for food and feed uses, import and processing under Regulation (EC) No 1829/2003. EFSA Journal 2017;15(3):4719, 31 pp. https://doi.org/10.2903/j.efsa.2017.4719

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Birch AN, Casacuberta J, De Schrijver A, Gralak MA, Jones H, Manachini B, Mess ean A, Nielsen EE, Nogu e F, Robaglia C, Rostoks N, Sweet J, Tebbe C, Visioli F, Wal J-M, Alvarez F, Ardizzone M, Liu Y, Neri FM and Ramon M, 2017c. Scientific opinion on an application by Dow AgroSciences LLC (EFSA- GMO-NL-2012-106) for the placing on the market of genetically modified herbicide-tolerant soybean DAS-44406-6 for food and feed uses, import and processing under Regulation (EC) No 1829/2003. EFSA Journal 2017;15(3):4738, 33 pp. https://doi.org/10.2903/j.efsa.2017.4738

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Birch AN, Casacuberta J, De Schrijver A, Gralak MA, Guerche P, Jones H, Manachini B, Messean A, Nielsen EE, Nogue F, Robaglia C, Rostoks N, Sweet J, Tebbe C, Visioli F, Wal J-M, Alvarez F, Ardizzone M, Mestdagh S and Ramon M, 2017d. Scientific opinion on an application for renewal of authorisation for continued marketing of maize 1507 and derived food and feed submitted under Articles 11 and 23 of Regulation (EC) No 1829/2003 by Pioneer Overseas Corporation and Dow AgroSciences LLC. EFSA Journal 2017;15(1):4659, 11 pp. https://www.efsa.europa.eu/en/efsajournal/pub/4659

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Bresson J-L, Dalmay T, Dewhurst IC, Epstein MM, Firbank LG, Guerche P, Hejatko J, Moreno FJ, Mullins E, Nogue F, Rostoks N, Sanchez Serrano JJ, Savoini G, Veromann E, Veronesi F, Devos Y, Ardizzone M, Neri FM, Papadopoulou N, De Sanctis G, Dumont AF, Gennaro A, Gomez Ruiz JA and Paraskevopoulos K, 2018. Scientific Opinion on the assessment of genetically modified maize MZHG0JG for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (application EFSA-GMO-DE-2016-133). EFSA Journal 2018;16(11):5469, 26 pp. https://doi.org/10.2903/j.efsa.2018.5469

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Bresson J-L, Dalmay T, Dewhurst IC, Epstein MM, Firbank LG, Guerche P, Hejatko J, Moreno FJ, Mullins E, Nogue F, Rostoks N, Sanchez Serrano JJ, Savoini G, Veromann E, Veronesi F, Alvarez F, Ardizzone M, De Sanctis G, Devos Y, Dumont AF, Gennaro A, Gomez Ruiz JA, Lanzoni A, Neri FM, Papadopoulou N, Paraskevopoulos K and Raffaello T, 2020. Scientific Opinion on the assessment of genetically modifiedmaize MZIR098 for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-DE-2017-142). EFSA Journal 2020;18(6):6171, 28 pp. https://doi.org/10.2903/j.efsa.2020.6171

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Bresson J-L, Dalmay T, Dewhurst IC, Epstein MM, Firbank LG, Guerche P, Hejatko J, Moreno FJ, Mullins E, Nogue F, Rostoks N, Sànchez Serrano JJ, Savoini G, Veromann E, Veronesi F and Fernandez Dumont A, 2021a. Statement on in vitro protein digestibility tests in allergenicity and protein safety assessment of genetically modified plants. EFSA Journal 2021;19(1):6350, 16 pp. https://doi.org/10.2903/j.efsa.2021.6350

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Bresson J-L, Dalmay T, Dewhurst IC, Epstein MM, Firbank LG, Guerche P, Hejatko J, Moreno FJ, Mullins E, Nogue F, Rostoks N, Sanchez Serrano JJ, Savoini G, Veromann E, Veronesi F, Alvarez F, Ardizzone M, De Sanctis G, Devos Y, Fernandez A, Gennaro A, Gomez Ruiz JA, Lanzoni A, Neri FM, Papadopoulou N, Paraskevopoulos K and Raffaello T, 2021b. Scientific Opinion on the assessment of genetically modified maize 1507 x MIR162 x MON810 x NK603 and subcombinations, for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL2015-127). EFSA Journal 2021;19(1):6348, 40 pp. https://doi.org/10.2903/j.efsa.2021.6348

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Mullins E, Bresson J-L, Dalmay T, Dewhurst IC, Epstein MM, Firbank LG, Guerche P, Hejatko J, Naegeli H, Moreno FJ, Nogue F, Rostoks N, Sanchez Serrano JJ, Savoini G, Veromann E, Veronesi F, Ardizzone M, De Sanctis G, Fernandez Dumont A, Federici S, Gennaro A, Gomez Ruiz AJ, Kagkli DM, Lanzoni A, Neri FM, Papadopoulou N, Paraskevopoulos K and Raffaello T, 2021c. Scientific Opinion on the Assessment of genetically modified maize NK603 x T25 x DAS-40278-9 and subcombinations, for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2019-164). EFSA Journal 2021;19(12):6942, 35 pp. https://doi.org/10.2903/j.efsa.2021.6942

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Mullins E, Bresson J-L, Dalmay T, Dewhurst IC, Epstein MM, Firbank LG, Guerche P, Hejatko J, Moreno FJ, Naegeli H, Nogue F, Rostoks N, Sanchez Serrano JJ, Savoini G, Veromann E, Veronesi F, Ardizzone M, Camargo AM, De Sanctis G, Federici S, Fernandez Dumont A, Gennaro A, Gomez Ruiz JA, Goumperis T, Kagkli DM, Lanzoni A, Lewandowska A, Lenzi P, Neri FM, Papadopoulou N, Paraskevopoulos K, Piffanelli P, Raffaello T, Streissl F, 2022a. Scientific Opinion on the assessment of genetically modified Maize MON 87429 for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2019-161). EFSA Journal 2022;20(11):7589, 40 pp. https://doi.org/10.2903/j.efsa.2022.7589

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Mullins E, Bresson J-L, Dalmay T, Dewhurst IC, Epstein MM, Firbank LG, Guerche P, Hejatko J, Naegeli H, Moreno FJ, Nogue F, Rostoks N, Sanchez Serrano JJ, Savoini G, Veromann E, Veronesi F, Ardizzone M, Dumont AF, Federici S, Gennaro A, Gomez Ruiz J A, Goumperis T, Kagkli DM, Lanzoni A, Lenzi P, Neri FM, Papadopoulou N, Paraskevopoulos K, Raffaello T, Streissl F and De Sanctis G, 2022b. Scientific Opinion on the assessment of genetically modified maize DP4114 x MON 810 x MIR604 x NK603 and subcombinations, for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2018-150). EFSA Journal 2022;20(3):7134, 38 pp. https://doi.org/10.2903/j.efsa.2022.7134

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Mullins E, Bresson J-L, Dalmay T, Dewhurst IC, Epstein MM, Firbank LG, Guerche P, Hejatko J, Naegeli H, Moreno FJ, Nogue F, Rostoks N, Sanchez Serrano JJ, Savoini G, Veromann E, Veronesi F, Ardizzone M, Camargo AM, De Sanctis G, Fernandez A, Gennaro A, Gomez Ruiz JA, Goumperis T, Kagkli DM, Neri FM, Raffaello T and Streissl F, 2022c. Scientific Opinion on the assessment of genetically modified maize MON 95379 for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2020-170). EFSA Journal 2022;20(11):7588, 31 pp. https://doi.org/10.2903/j.efsa.2022.7588

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2012. Scientific Opinion on Dietary Reference Values for protein. EFSA Journal 2012;10(2):2557, 66 pp. https://doi.org/10.2903/j.efsa.2012.2557

EFSA Scientific Committee, 2011. EFSA guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed. EFSA Journal 2011;9(12):2438, 21 pp. https://doi.org/10.2903/j.efsa.2011.2438

Ferraro DJ, Gakhar L and Ramaswamy S, 2005. Rieske business: Structure-function of Rieske non-heme oxygenases. Biochemical and Biophysical Research and Communications, 338, 175–190.

Foo ACY and Mueller GA, 2021. Abundance and stability as common properties of allergens. Frontiers in Allergy, 2, 769728. https://doi.org/10.3389/falgy.2021.769728

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

Helm RM, 2001. Topic 5: Stability of Known Allergens (Digestive and Heat Stability). Report of a joint FAO, WHO expert consultation on allergenicity of food derived from biotechnology, 22–25 January 2001.

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

Krueger JP, Butz RG, Atallah YH and Cork DJ, 1989. Isolation and identification of microorganisms for the degradation of dicamba. Journal of Agricultural and Food Chemistry, 37, 534–538.

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

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

Li Y, Tran AH, Danishefsky SJ and Tan Z, 2019. Chemical biology of glycoproteins: from chemical synthesis to biological impact. Methods in Enzymology, 621, 213–229.

Looney WJ, Narita M and Mühlemann K, 2009. Stenotrophomonas maltophilia: an emerging opportunist human pathogen. The Lancet Infectious Diseases, 9, 312–323. https://doi.org/10.1016/S1473-3099(09)70083-0

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

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

Palaudelmàs M, Peñas G, Melé E, Serra J, Salvia J, Pla M, Nadal A and Messeguer J, 2009. Effect of volunteers on maize gene flow. Transgenic Research, 18, 583–594.
Pascher K, 2016. Spread of volunteer and feral maize plants in Central Europe: recent data from Austria. Environmental Sciences Europe, 28, 30.
Polevoda B and Sherman F, 2000. Na-terminal acetylation of eukaryotic proteins. Journal of Biological Chemistry, 275, 36479–36482.
Qureshi A, Mooney L, Denton M and Kerr KG, 2005. Stenotrophomonas maltophilia in salad. Emerging Infectious Diseases, 11, 1157–1158.
Steinberg P, van der Voet H, Goedhart PW, Kleter G, Kok EJ, Pla M, Nadal A, Zeljenková D, Aláčová R, Babincová J, Rollerová E, Jad'ud'ová S, Kebis A, Szabova E, Tulinská J, Líšková A, Takácsová M, Mikušová ML, Krivošíková Z, Spök A, Racovita M, de Vriend H, Alison R, Alison C, Baumgärtner W, Becker K, Lempp C, Schmicke M, Schrenk D, Pöting A, Schiemann J and Wilhelm R, 2019. Lack of adverse effects in subchronic and chronic toxicity/carcinogenicity studies on the glyphosate-resistant genetically modified maize NK603 in Wistar Han RCC rats. Archives of Toxicology, 93, 1095–1139.
Steinberg P, van der Voet H, Goedhart PW, Kleter G, Kok EJ, Pla M, Nadal A, Zeljenková D, Aláčová R, Babincová J, Rollerová E, Jad'ud'ová S, Kebis A, Szabova E, Tulinská J, Líšková A, Takácsová M, Mikušová ML, Krivošíková Z, Racovita ASM, de Vriend H, Alison R, Alison C, Baumgärtner W, Becker K, Lempp C, Schmicke M, Schrenk D, Pöting A, Schiemann J and Wilhelm R, 2020. Correction to lack of adverse effects in subchronic and chronic toxicity/carcinogenicity studies on the glyphosate-resistant genetically modified maize NK603 in Wistar Han RCC rats. Archives of Toxicology, 94, 1779–1781.
Sys C, Van Ranst E, Debaveye J and Beernaert F, 1993. Land evaluation. Part III: Crop requirements. Agricultural Publication No. 7. Brussels, General Administration for Development Cooperation. 199 pp.
Trtikova M, Lohn A, Binimelis R, Chapela I, Oehen B, Zemp N, Widmer A and Hilbeck A, 2017. Teosinte in Europe – searching for the origin of a novel weed. Scientific Reports, 71, 1560.
Wang R, Glenn KC, Kessenich C, Bell E, Burzio LA and Koch MS, 2016. Li B and Silvanovich A, 2016. Safety assessment of dicamba mono-oxygenases that confer dicamba tolerance to various crops. Regulatory Toxicology and Pharmacology, 81, 171–182.
Windels P, Alcalde E, Lecoq E, Legris G, Pleysier A, Tinland B and Wandelt C, 2008. General surveillance for import and processing: the EuropaBio approach. Journal of Consumer Protection and Food Safety, 3(S2), 14–16.
Wu G, Bazer FW, Dai Z, Li D, Wang J and Wu Z, 2014. Amino acid nutrition in animals: protein synthesis and beyond. Annual Review of Animal Biosciences, 2, 387–417. https://doi.org/10.1146/annurev-animal-022513-114113

Abbreviations

ADF	acid detergent fibre
bp	base pair
bw	body weight
CTP	chloroplast transit peptide
DMO	dicamba mono-oxygenase
dw	dry weight
ELISA	enzyme-linked immunosorbent assay
ERA	environmental risk assessment
FOB	functional observational battery
FMV	figwort mosaic virus
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
NDF	neutral detergent fibre
NGS	next generation sequencing
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PAT	phosphinothricin acetyltransferase
PCR	polymerase chain reaction
PMEM	post-market environmental monitoring
RB	right border

SDS–PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SES	standardised effect sizes
T-DNA	transfer-deoxyribonucleic acid
TEV	tobacco etch virus
UTR	untranslated region

Appendix A – Additional studies

List of additional studies performed by or on behalf of the applicant with regard to the evaluation of the safety of maize MON 87419 for humans, animal or the environment

Study identification	Title
PLC-2013-0606/ MSL0026134	Amended Report for MSL0026040: Dormancy and Germination Evaluation of Maize MON 87419 Using Seed Produced at U.S. Field Sites
PLC-2013-0173/ MSL0025678	Pollen Viability and Morphology Evaluation of Maize MON 87419 Grown in a 2013 U.S. Field Trial.
MSL0025672[(a)]	Evaluation of Maize grain from MON 87419 as a Feed Ingredient for Channel Catfish.
MSL0026257[(a)]	Comparison of Broiler Performance and Carcass Parameters When Fed Diets Containing MON 87419, Control, or Reference Maize Grain.
SCR-2014-0341[(a)]	Comparison of Broiler Performance and Carcass Parameters When Fed Diets Containing MON 87419, Control, or Reference Maize Grain. By Gender Analysis
MSL0025676	Amended Report for MSL0025610: Assessment of DMO and PAT Protein Levels in Maize Tissues Collected from MON 87419 Produced in United States Field Trials. During 2013.
SCR-2016-0235	Characterization of the Dicamba Mono-Oxygenase Protein Purified from the Maize Grain of MON 87419 and *Escherichia coli* (*E. coli*)-Produced MON 87419 Dicamba Mono-Oxygenase Protein
SCR-2016-0241	Characterization of the Phosphinothricin N-Acetyltransferase (pat) Protein Purified from the Maize Grain of MON 87419 and *Escherichia coli* (*E. coli*)-Produced PAT (*pat*)
SCR-2016-0322	N-terminal sequence analysis of the Dicamba Mono-Oxygenase Protein Purified from the Maize Grain of MON 87419.
MSL0025656	PCR Analysis to Confirm the Absence of *Agrobacterium tumefaciens* Used to Produce MON 87419
MSL0025848	Molecular Characterization of MON 87419 Maize by Southern Blot Analysis
MSL0025992	Comparison of Lipid Transfer Protein (LTP) Expression Levels from MON 87419 and Conventional Control Maize

(a): The GMO Panel notes that the submitted study report contained limited details about the materials and methods used for the production of the test diets. As the study was not a requirement for the EU, clarification of the limitations was not sought. On evaluation of the available information, no treatment-related adverse effects were identified.

Appendix B – List of relevant publications identified by the applicant through literature searches (January 2007–June 2022)

Reference
Canadian Food Inspection Agency, 2022: Decision document DD2016-113: Determination of the safety of Monsanto Canada Inc.'s corn (*Zea mays* L.) event MON 87419 https://inspection.canada.ca/plant-varieties/plants-with-novel-traits/approved-under-review/decision-documents/dd2016-113/eng/1505250753854/1505250754313
Food Standards Australia New Zealand, 2022: A1118 – Food derived from Herbicide-tolerant Corn Line MON87419 https://www.foodstandards.gov.au/code/applications/Pages/A1118GM-CornLineMON87419.aspx
Health Canada, 2022: Herbicide Tolerant Maize – MON 87419 https://www.canada.ca/en/health-canada/services/food-nutrition/genetically-modified-foods-other-novel-foods/approved-products/herbicide-tolerant-maize-mon-87419.html
Ministry of Agriculture, Forestry and Fisheries, 2022. 除草剤ジカンバ及びグルホシネート耐性トウモロコシ(改変dmo, pat, *Zea mays* subsp. *mays* (L.) Iltis) (MON87419, OECD UI: MON-87419-8)【ﾊｲｴﾙｸﾛｯﾌﾟｻｲｴﾝｽ株式会社】 https://www.maff.go.jp/j/syouan/nouan/carta/torikumi/attach/pdf/index-23.pdf
United States Food and Drug Administration, 2022: MON 87419 (Herbicide tolerance (dicamba), Herbicide Tolerance (glufosinate ammonium)). https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=NewPlantVarietyConsultations&id=MON-87419-8

Appendix C – Statistical analysis of the 90-day study on maize MON 87419 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, functional observational battery (FOB) data, locomotor activity data and histopathology data (microscopic findings). 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 continuous 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 and a part of the locomotor activity parameters) 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 food consumption, clinical pathology parameters and organ weights (absolute and relative), FOB and motor activity evaluations, 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.1: Statistically significant findings in 90-day study on maize MON 87419 in rats

Statistically significant parameter/endpoint	Finding	GMO Panel interpretation
Food consumption	Increased (4%) in top dose females weeks 1–2.	Small magnitude. No effect on body weight. Not an adverse effect of treatment.
Hindlimb and forelimb grip strength	Decreased 20% in the low dose groups combined.	No significant effect at the high dose level. Within normal variation. Not an adverse effect of treatment.
Hindlimb footsplay	Decreased (10%) in males of both groups. Increased (15%) in females.	Small magnitude. No consistency across sexes. Within normal variation. Not an adverse effect of treatment.
Cholesterol	Decreased (10%) at the top dose level in both sexes combined.	Small magnitude. Not adverse in isolation. No associated findings. Not an adverse effect of treatment.
Liver weight relative to body weight	Decreased (5%) in low dose males.	Small magnitude. Within normal variation. No associated histopathological or clinical chemistry findings. Not an adverse effect of treatment.
Testes weights (absolute and relative to body weight)	Decreased (8%) at low dose.	Small magnitude. Within normal variation. No associated histopathological findings. Not an adverse effect of treatment.
Ovary / oviduct weights (absolute and relative to body weight)	Increased (12%) at top dose.	Small magnitude. Within normal variation. No associated histopathological findings. Not an adverse effect of treatment.
Pituitary weights	Increased (15%) in low dose groups combined.	Small magnitude. Not present at top dose level. No associated histopathological findings. Not an adverse effect of treatment.
Thymus weights	Increased (16%) in low dose females.	Small magnitude. Not present at top dose level. No associated histopathological or haematology findings. Not an adverse effect of treatment.

Appendix D – Animal dietary exposure

Table D.1: Animal dietary exposure to PAT and DMO proteins (μg/kg bw per day) based on the consumption of maize grains, gluten feed and gluten meal

Animal species BW (kg)/ total diet intake (kg dw)	Feed material	IR%	PAT	DMO
Broiler 1.7/0.12	Grain	70	45.95	9.38
	Gluten feed	10	17.08	3.45
	Gluten meal	10	46.58	9.52
	Total	90	110	22
Finishing pig 100/3	Grain	70	19.53	3.99
	Gluten feed	20	14.52	2.94
	Gluten meal	10	19.8	4.05
	Total	100	54	11
Lactating dairy cow 650/25	Grain	30	10.73	2.19
	Gluten feed	30	27.92	5.65
	Gluten meal	20	50.76	10.38
	Total	100	89	18

Table D.2: Animal dietary exposure to PAT and DMO proteins (μg/kg bw per day) based on the consumption of maize forage

Animal species BW (kg)/ total diet intake (kg dw)	Feed material	IR%	PAT	DMO
Beef 500/12	Forage	80	96	115.20
Lactating dairy cow 650/25	Forage	60	115.38	138.46
lamb 40/1.7	Forage	30	63.75	76.50
breeding swine 260/6	Forage	20	23.07	27.69
Layer 1.9/0.13	Forage	10	34.21	41.05