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SCIENTIFIC OPINION

Assessment of genetically modified maize MON 94804 (application GMFF-2022-10651)

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Abstract

Genetically modified (GM) maize MON 94804 was developed to achieve a reduction in plant height by introducing the *GA20ox_SUP* suppression cassette. The molecular characterisation and bioinformatic analyses do not identify issues requiring food/feed safety assessment. None of the agronomic/phenotypic and compositional differences identified between maize MON 94804 and its conventional counterpart needs further assessment, except for ear height, plant height and levels of carbohydrates in forage, which do not raise safety or nutritional concerns. The Panel on Genetically Modified Organisms (GMO Panel) does not identify safety concerns regarding the toxicity and allergenicity of the GA20ox_SUP precursor-miRNA and derived mature miRNA as expressed in maize MON 94804 and finds no evidence that the genetic modification would change the overall allergenicity of maize MON 94804. In the context of this application, the consumption of food and feed from maize MON 94804 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that maize MON 94804 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 94804 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 94804. The GMO Panel concludes that maize MON 94804 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

GA20ox, genetic engineering, GM, import and processing, maize (*Zea mays*), MON 94804

CONTENTS

SUMMARY

Following the submission of application GMFF-2022-10651 under Regulation (EC) No 1829/2003 from Bayer Agriculture BV (referred to hereafter as 'the applicant'), the Panel on Genetically Modified Organisms of the European Food Safety Authority (referred to hereafter as 'GMO Panel') was asked to deliver a Scientific Opinion on the safety of genetically modified (GM) reduced-height maize (*Zea mays* L.) MON 94804 according to Regulation (EU) No 503/2013. The scope of application GMFF-2022-10651 is for import, processing and food and feed uses of maize MON 94804 within the European Union (EU) 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 94804 according to the scope of the application GMFF-2022-10651. The GMO Panel conducted the assessment of maize MON 94804 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 94804 contains a single insert consisting of one copy of the *GA20ox_SUP* suppression cassette. Bioinformatics analyses of the newly created open reading frames within the insert or spanning the junctions between the insert and genomic DNA do not raise any safety concerns. The *in planta* RNA interference (RNAi) off-target search, performed with the sequence of the GA20ox_SUP microRNA (miRNA), does not provide indication of an off-target effect that would require further safety assessment. The stability of the inserted DNA and of the introduced trait is confirmed over several generations.

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 differences identified in agronomic, phenotypic and compositional characteristics between maize MON 94804 and its conventional counterpart needs further consideration except for ear height, plant height and levels of carbohydrates in forage, which do not raise concerns when assessed for food/feed and environmental safety. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the GA20ox_SUP precursor-miRNA and derived mature miRNA as expressed in maize MON 94804. The GMO Panel finds no evidence that the genetic modification would change the overall safety of maize MON 94804. In the context of this application, the consumption of food and feed from maize MON 94804 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that maize MON 94804 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 trait, the outcome of the agronomic and phenotypic analysis and the routes and levels of exposure, maize MON 94804 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 94804.

The literature searches did not identify any relevant publications on maize MON 94804; therefore, the GMO Panel does not identify any safety issue pertaining to the intended uses of maize MON 94804.

The GMO Panel concludes that maize MON 94804 is as safe as its conventional counterpart and the tested non-GM maize reference varieties with respect to potential effects on human and animal health and the environment.

1 | INTRODUCTION

The scope of the application GMFF-2022-10651 is for food and feed uses, import and processing of maize MON 94804 and does not include cultivation in the European Union (EU). Maize MON 94804 was developed to reduce internode length in the maize stalk and to obtain, as a result, a reduction in plant height.

1.1 | Background

On 14 February 2023, the European Food Safety Authority (EFSA) received from the Competent Authority of The Netherlands application GMFF-2022-10651 for authorisation of maize MON 94804 (Unique Identifier MON-948Ø4-4), submitted by Bayer Agriculture BV (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003.[1] Following receipt of application GMFF-2022-10651, EFSA informed EU Member States (MS) and the European Commission, and made the application available to them. Simultaneously, EFSA published a summary of the application.[2]

EFSA checked the application for compliance with the relevant requirements of Regulation (EC) No 1829/2003, Regulation (EU) No 503/2013[3] and the EFSA guidance documents and, when needed, asked the applicant to supplement the initial application. On 2 May 2023, EFSA declared the application valid.

From validity date, EFSA and the EFSA Panel on Genetically Modified Organisms (referred to hereafter as 'GMO Panel') endeavoured to respect a time limit of 6 months to issue a scientific opinion on application GMFF-2022-10651. Such time limit was extended whenever EFSA and/or its GMO Panel requested supplementary information from 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 as of date of validity to make their opinion on application GMFF-2022-10651 known.

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 94804 in the context of its scope as defined in application GMFF-2022-10651.

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

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a confidential and a non-confidential version of the dossier GMFF-2022-10651 following the EFSA requirements as detailed in and EFSA (2021a; 2021b).

In accordance with Art. 38 of the Regulation (EC) No 178/2002 and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation,[6] and the Decision of EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality, the non-confidential version of the dossier has been published on OpenEFSA.[7] According to Art. 32c (2) of Regulation (EC) No 178/2002[8] and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations,[9] EFSA carried out a public consultation on the non-confidential version of the dossier from 26 September to 17 October 2023 for which no comments were received.

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

[2]Available online: https://open.efsa.europa.eu/dossier/GMFF-2022-10651?type=node&key=1109066

[3]Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. OJ L157, 8.6.2013, p. 1–48.

[4]Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 12.3.2001, p. 1–38.

[5]These particulars are available online at: https://open.efsa.europa.eu/study-inventory/EFSA-Q-2023-00106

[6]Decision available at: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements

[7]https://open.efsa.europa.eu/questions/EFSA-Q-2023-00106

[8]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–48.

[9]Decision available at: https://www.efsa.europa.eu/sites/default/files/corporate_publications/files/210111-PAs-pre-submission-phase-and-public-consultations.pdf

The GMO Panel based its scientific assessment of maize MON 94804 on the valid dossier GMFF-2022-10651, additional information provided by the applicant during the risk assessment, scientific comments submitted by EU MS and relevant peer-reviewed scientific publications.

2.2 | Methodologies

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

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

3 | ASSESSMENT

3.1 | Introduction

MON 94804 maize expresses the GA20ox_SUP microRNA (miRNA), which downregulates the expression of two endogenous gibberellic acid 20 oxidase (GA20ox) genes, *GA20ox3* and *GA20ox5*. The suppression of the two target genes results in a decrease in gibberellic acid levels in the maize stalk, which leads to a reduced internode length in the stalk and, as a consequence, to a reduction in plant height.

3.2 | Systematic literature review

The GMO Panel assessed the applicant's literature searches on maize MON 94804, which include a scoping review, according to the guidelines given in EFSA (2010; 2019b).

A systematic review as referred to in Regulation (EU) No 503/2013 has not been provided in support to the risk assessment of application GMFF-2022-10651. Based on the outcome of the scoping review, the GMO Panel agrees that there is limited value in undertaking a systematic review for MON 94804 maize at present.

The GMO Panel considered the overall quality of the performed literature searches acceptable. The literature searches did not identify any relevant publications on maize MON 94804; therefore, the GMO Panel does not identify any safety issue pertaining to the intended uses of maize MON 94804.

3.3 | Molecular characterisation[10]

3.3.1 | Transformation process and vector constructs

Maize MON 94804 was developed by a two-step process. In the first step, mature embryos of maize inbred line HCL301 were co-cultured with a disarmed *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*) strain ABI containing the vector PV-ZMAP527829. In the second step, selected R2 lines were crossed with maize inbred line HCL301, which had been transformed with vector PV-ZMOO513642, allowing the expression of Cre recombinase. In the resulting plants, the *cp4 epsps* cassette was excised by the Cre recombinase, and the *cre* gene was subsequently segregated away, through conventional breeding, to obtain maize MON 94804. The plasmid PV-ZMAP527829 used for the transformation contains two expression cassettes between the right and left border of the transfer-deoxyribonucleic acid (T-DNA), containing the following genetic elements:

- The *cp4 epsps* expression cassette consisting of the promoter and leader sequence of actin 1 gene (*R-act1*) from *Oryza sativa*, the chloroplast transit peptide (TS-CTP2) of the *shkG* gene from *Arabidopsis thaliana*, the coding sequence of the *aroA* gene from *A*. sp. encoding the CP4 EPSPS protein and the 3′ untranslated sequence of the nopaline synthase (*nos*) gene from *A*. *tumefaciens*. The cassette is flanked by *loxP* sites from bacteriophage P1.
- The *GA20ox_SUP* suppression cassette consisting of the promoter and leader from the rice tungro bacilliform virus (RTBV), the intron and flanking exon sequence of the *hsp70* gene from *Zea mays*, the sequence of the precursor RNA for miR1425 from rice where the 21 nt mature miRNA and the 21 nt reverse complementary passenger strand of the mature

[10]Dossier: Part II – Section 1.2; additional information: 10/01/2024.

miRNA have been replaced with sequences specific to *GA20ox3* and *GA20ox5* genes from *Zea mays*, and the 3′ untranslated region (UTR) from multiple 3′ UTR sequences from *Zea mays*.

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

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

- The *cre* expression cassette consisting of the promoter, leader, intron and flanking 5′ untranslated sequence of the *act1* gene from *O. sativa*, two partial regions of the *cre* recombinase gene from bacteriophage P1 interrupted by the second intron sequence from the light inducible (*LS1*) gene from *Solanum tuberosum* and the 3′ untranslated sequence of the *Hsp17* gene from *Triticum aestivum*.
- The *nptII* cassette consisting of the 35S promoter and leader sequence from the cauliflower mosaic virus (CaMV), the coding sequence of the *nptII* gene from *Escherichia coli* and the 3′ untranslated sequence of the *nos* gene from *A. 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 94804 was performed by next generation sequencing (NGS) and junction sequence analysis (JSA) in order to determine insert copy number, to confirm the absence of PV-ZMAP527829 plasmid backbone and the entire PV-ZMOO513642 plasmid, and by sequencing on polymerase chain reaction (PCR) amplified fragments to determine size and organisation of the inserted sequences.

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

NGS/JSA of the whole genome indicated that maize MON 94804 contains a single insert, consisting of a single copy of the T-DNA in the same configuration as in the PV-ZMAP527829 transformation vector with the exception that *cp4 epsps* and one *loxP* site are absent as intended. NGS/JSA also confirmed the absence of plasmid backbone sequences in the maize genome.

The nucleotide sequence of the entire insert of maize MON 94804 together with 1000 bp of the 5′ and 1000 bp of the 3′ flanking regions was determined. The insert of 2733 bp is identical to the T-DNA of the PV-ZMAP527829 vector, with the exceptions of the removed *cp4 epsps* cassette and the border regions: The entire right border region (330 bp) is absent in maize MON 94804, whereas the 5′ end of the left border is deleted, with only 40 bp remaining identical to the PV-ZMAP527829 plasmid.

A comparison with the pre-insertion locus indicated that 41 bp were deleted from the maize genomic DNA. The possible interruption by the insertion of known endogenous maize genes in maize MON 94804 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 94804.

The results of segregation (see Section 3.2.5) and bioinformatic analyses are compatible with a single insertion in the nuclear genome.

Bioinformatic analyses of the newly created open reading frames (ORFs) within the insert and spanning the junctions between the insert and genomic DNA revealed that one short ORF presented an 8-amino acid exact match to known allergens. However, this ORF is in the reverse orientation and lacks any start codon and known promoter element. No significant similarities with toxins were identified for any ORF within the insert and spanning the junctions between the insert and genomic DNA. In conclusion, these analyses indicated that the expression of any ORF showing significant similarities to toxins or allergens in maize MON 94804 is unlikely.

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 94804 to microbial DNA. The likelihood and potential consequences of plant to bacteria gene transfer are described in Section 3.5.1.2.

According to Regulation (EU) No 503/2013, when silencing approaches by RNA interference (RNAi) have been used in GM plant applications, a bioinformatic analysis to identify potential 'off target' genes is required. The applicant has followed the recommendations by the GMO Panel for an RNAi off-target search in maize MON 94804 expressing the GA20ox_SUP miRNA.[11] Except for the expected target sequences of gibberellin 20-oxidase 3 and gibberellin 20-oxidase 5, none of the maize transcript sequences present in the available databases showed similarity to the mature GA20ox_SUP miRNA sequence. The GMO Panel assessed this information and concluded that it does not provide indication of an off-target effect of the GA20ox_SUP miRNA expression that would need further safety assessment.

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

3.3.3 | Protein characterisation and equivalence

Maize MON 94804 does not express any newly expressed proteins.

3.3.4 | Information on the expression of the insert

In the absence of newly expressed proteins, no protein level data was provided by the applicant.

According to the data provided by the applicant, GA20ox_SUP miRNA downregulates the expression of *GA20ox3 and GA20ox5*, the products of which are involved in the biosynthetic pathway of gibberellic acid. The applicant provided a comparative analysis of the relative mRNA levels of the target genes (*GA20ox3* and *GA20ox5*) and the levels of the major bioactive gibberellins (GA1 and GA4) in maize MON 94804 and its conventional counterpart. The results indicate that in the GM plants, the expression of both target genes was reduced in several tissues (over season leaf (OSL) 1, OSL4, stalk, silk and grain for *GA20ox3*; OSL1, OSL4, stalk and grain for *GA20ox5*), and gibberellin levels were also reduced in several tissues (leaf and forage for GA1; starting seed,[12] leaf, stalk and forage for GA4). These results support the expected mode of action of GA20ox_SUP miRNA.

3.3.5 | Inheritance and stability of inserted DNA

Genetic stability of maize MON 94804 insert was assessed using NGS to sequence the insert and the flanking regions from five generations (F4, F4F1, F5, F5F1 and F6) while segregation analysis was performed by PCR-based analysis from three consecutive generations (F4F2, F4F3 and F4F4). The results indicate that all the plants tested retained a single copy of the insert and flanking regions, which were stably inherited in subsequent generations. The results support the presence of a single insertion, segregating in a Mendelian fashion.

3.3.6 | Conclusion on molecular characterisation

The molecular characterisation data establish that maize MON 94804 contains a single insert consisting of one copy of the *GA20ox_SUP* suppression cassette. Bioinformatic analyses of the newly created ORFs within the insert or spanning the junctions between the insert and genomic DNA do not raise any safety concerns. The *in planta* RNAi off-target search, performed with the sequence of the GA20ox_SUP miRNA, does not provide indication of an off-target effect that would require further safety assessment. The stability of the inserted DNA and of the introduced trait is confirmed over several generations.

3.4 | Comparative analysis[13]

3.4.1 | Overview of studies conducted for the comparative analysis

Application GMFF-2022-10651 presents data on agronomic and phenotypic characteristics, as well as on forage and grain composition of maize MON 94804 (Table 1). In addition, the application includes a germination and viability study of the maize line containing event MON 94804.

TABLE 1 Main comparative analysis studies to characterise maize MON 94804 provided in application GMFF-2022-10651.

Study focus	Study details	Comparator	Non-GM reference varieties
Agronomic and phenotypic analysis Compositional analysis	Field study, U.S., 2020, eight sites[a]	HCL301 × HCL617	19[b]

Abbreviation: GM, Genetically modified.

[a]The field trials were located in Shelby, IA; Jefferson, IA; Warren IL; Shelby, IL; Stark, IL; Clinton, IN; York, NE and Miami, OH.

[b]The non-GM hybrids with their corresponding comparative relative maturity indicated in brackets were Dekalb DKC61-52 (111); Dekalb DKC62-06 (112); Dekalb DKC64-85 (114); Dekalb DKC65-18 (115); Fontanelle 10C616 (110); Golden Harvest G07F23 (107); Golden Harvest G10T63 (110); Hubner H3624 (113); Lewis 1312 (112); Mycogen 2H721 (112); Pioneer P0993 (109); Pioneer P1105 (111); Pioneer P1345 (113); Stewart S480 (106); Stewart S660 (113); Stewart S750 (114); Stine 9654-0 (107); Stine 9714-0 (109) and Stone 5420 (113).

[12]The starting seed was from the same lot as in the field trial study, see Section 3.4.3.

[13]Dossier: Part II – Section 1.3; additional information: 28/7/2023.

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 94804, the comparator HCL301 × HCL617 and four non-GM reference varieties.

The agronomic, phenotypic and compositional data were analysed as specified by EFSA GMO Panel (2010b; 2011a). This includes the application of a difference test (between the GM maize and the non-GM comparator) and an equivalence test (between the GM maize and the set of non-GM commercial reference varieties). The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).[14]

3.4.3 | Suitability of selected test materials

3.4.3.1 | *Selection of the test materials*

As described in Section 3.3.1, inbred line HCL301 was transformed to obtain line MON 94804, which was then crossed with inbred line HCL617 to produce the hybrid maize MON 94804[15] used in the comparative analysis.

The comparator used in the field trials is the non-GM maize hybrid HCL301 × HCL617, which is isogenic to hybrid maize MON 94804 (as documented by the pedigree) and is considered to be the conventional counterpart.

Both hybrid maize MON 94804 and the conventional counterpart have a comparative relative maturity (CRM) of 111, which is considered appropriate for growing in environments across the US, where the comparative field trials were conducted.

Commercial non-GM reference varieties with a CRM ranging from 106 to 115 were selected by the applicant and, at each selected site, four reference varieties were tested (see Table 1). On the basis of the information provided on relative maturity classes, the GMO Panel considers the selected non-GM reference varieties appropriate for the comparative assessment.

3.4.3.2 | *Seed production and quality*

The seeds of maize MON 94804 and of the conventional counterpart used in the 2020 field trials (see Table 1) were produced, harvested and stored under similar conditions. The seed lots were verified for their identity via event-specific PCR analysis. The germination of maize MON 94804 and the conventional counterpart was tested under optimal and suboptimal temperatures.[16] The germination capacity of maize MON 94804 was compared with that of its conventional counterpart and four reference maize hybrids.[17] The results of these studies indicate that there was no difference in seed germination between maize MON 94804 and its conventional counterpart.[18]

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

3.4.3.3 | *Conclusion on suitability*

The GMO Panel considers that maize MON 94804, the conventional counterpart, and the non-GM 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 US. The soil and climate characteristics of the selected fields[19] correspond to optimal, near optimal and sub-optimal conditions for maize cultivation (Sys et al., 1993). Despite a limited variability of soil characteristics, the GMO Panel considers that the selected sites reflect the commercial maize-growing regions in which the test materials are likely to be grown.

[14]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).

[15]For the agronomic, phenotypic and compositional analysis, hybrid maize MON 94804 refers to the event obtained crossing inbred line MON 94804 HCL301 with inbred line HCL617.

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

[17]These were: Dekalb DKC64-85; Stine 9654-0; Pioneer P0993; and Mycogen 2H721.

[18]The GM hybrid maize showed a mean germination of 99.8% and 99% under optimal and suboptimal temperatures respectively, while the conventional counterpart showed a mean of 99% under both temperatures.

[19]Soil types of the field trials were silty clay loam and silt loam (covering only optimal conditions); soil organic carbon ranged from 1.3% to 3.3% (covering only optimal conditions); soil pH ranged from 6.3 to 7.1 (covering optimal and near-optimal conditions). Average temperatures and sum of precipitations during the usual crop growing season ranged respectively from 18.2°C to 21.7°C and from 307 mm to 768 mm (covering optimal, near-optimal and sub-optimal conditions).

3.4.4.2 | *Meteorological conditions*

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

3.4.4.3 | *Management practices*

The field trials included plots containing maize MON 94804, plots with the conventional counterpart and plots with non-GM maize reference varieties, mostly managed according to local agricultural practices. Despite not being considered a normal agricultural practice, thinning was applied at four field trial sites[20] to achieve a more homogeneous plant density across plots. The GMO Panel considers that the management practices including sowing, harvesting and application of plant protection product were acceptable for the selected receiving environments.

3.4.4.4 | *Conclusion on representativeness*

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

3.4.5 | Agronomic and phenotypic analysis

Eleven agronomic and phenotypic endpoints[21] plus information on abiotic stressors, disease incidence and arthropod damage were collected from the field trial sites (see Table 1). The endpoint fruit count was not analysed with formal statistical methods because of lack of variability in the data.

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

- For maize MON 94804, the test of difference identified statistically significant differences with the conventional counterpart for seven endpoints (days to flowering, ear height, plant height, days to maturity, lodging, final stand count and moisture). All these endpoints fell under equivalence category I or II except for ear height and plant height, which fell under equivalence category IV.[22]

Ear height and plant height for maize MON 94804 were reduced with respect to the conventional counterpart and the reference varieties. This is expected because of the intended trait (see Section 3.1).

3.4.6 | Compositional analysis

Maize MON 94804 grain and forage harvested from eight sites (Table 1) were analysed for 78 constituents (nine in forage and 69 in grains), including those recommended by OECD (OECD, 2002). The statistical analysis was not applied to 15 grain constituents because their concentration in more than half of the samples was below the limit of quantification.[23]

The statistical analysis was applied to a total of 63 constituents (54 in grains[24] and nine in forage[25]); a summary of the outcome of the test of difference and the test of equivalence is presented in Table 2.

For maize MON 94804 statistically significant differences with the conventional counterpart were found for 19 endpoints (six in forage and 13 in grain). All the endpoints for which significant differences were found between maize MON 94804 and the conventional counterpart fell under equivalence category I or II, except for carbohydrates in forage which fell under equivalence category III.

[20]Jefferson, IA; Clinton, IN; York, NE and Miami, OH.

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

[22]For ear height, the estimated mean values (in cm) for the conventional counterpart, the GM maize and the reference varieties were 126.4, 69.4 and 115.9, respectively; the equivalence interval was 86.6-145.3. For plant height, the estimated mean values (in cm) for the conventional counterpart, the GM maize and the reference varieties were 252.6, 157.3 and 238.8, respectively; the equivalence interval was (183.4, 294.1).

[23]Caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), myristoleic acid (C14:1), pentadecanoic acid (C15:0), pentadecenoic acid (C15:1), heptadecanoic acid (C17:0), heptadecenoic acid (C17:1), γ-linolenic acid (C18:3), eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3), arachidonic acid (20:4), sodium and furfural.

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

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

TABLE 2 Outcome of the comparative compositional analysis in grain and forage for maize MON 94804. The table shows the number of endpoints in each category.

		Test of difference[a]	
		Not different	Significantly different
Test of equivalence[b]	Category I/II	43	18[c]
	Category III/IV	–	1[d]
	Not categorised	1[e]	–
	Total endpoints	63	

[a]Comparison between maize MON 94804 and its conventional counterpart.

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

[c]Endpoints with significant differences between maize MON 94804 and its conventional counterpart falling in equivalence categories I–II. In grain: total fat, total dietary fibre (TDF), calcium, magnesium, manganese, thiamine, lysine, palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), eicosenoic acid (C20:1), raffinose, ferulic acid; in forage: ash, protein, total fat, acid detergent fibre (ADF), neutral detergent fibre (NDF).

[d]Endpoints with significant differences between the MON 94804 and its conventional counterpart and falling in equivalence category III: carbohydrates in forage. Estimated means for this endpoint are reported in Table 3.

[e]Endpoints not categorised for equivalence and without significant differences between the MON 94804 and its conventional counterpart: acid detergent fibre (ADF) in grain.

The GMO Panel assessed all the significant differences between maize MON 94804 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 endpoint showing significant differences between maize MON 94804 and its conventional counterpart and falling under category III are given in Table 3.

TABLE 3 Quantitative results (estimated means and equivalence limits) for the compositional endpoint in forage that is further assessed based on the results of the statistical analysis.

			Non-GM reference varieties	
Endpoint	**Maize MON 94804**[a]	**Conventional counterpart**	**Mean**	**Equivalence limits**
Carbohydrates (% dw)	85.67*	86.72	87.10	85.84–88.36

Abbreviation: dw, dry weight.

[a]For maize MON 94804, significantly different values are marked with an asterisk, while the outcome of the test of equivalence is highlighted by a light grey background (equivalence category III).

According to the applicant, GA20ox_SUP miRNA down-regulates the expression of the *GA20ox3* and *GA20ox5*, the products of which are involved in the biosynthetic pathway of gibberellic acid. The applicant quantified the levels of gibberellins in different tissues (Section 3.3.4).

3.4.7 | Conclusion on comparative analysis

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

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 94804 and its conventional counterpart needs further assessment except for ear height and plant height, which are further assessed for potential environmental impact in Section 3.6.
- None of the differences identified in forage and grain composition between maize MON 94804 and its conventional counterpart needs further assessment regarding food and feed safety except for carbohydrates in forage, which is further assessed in Section 3.5.5.

3.5 | Food/feed safety assessment[26]

3.5.1 | Overview of overarching information for food/feed assessment

3.5.1.1 | *Compositional analysis*

The compositional analysis of maize MON 94804 and the conventional counterpart provided by the applicant and assessed by the GMO Panel is described in Section 3.4.6.

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

No newly expressed proteins are present in maize MON 94804. The GA20ox_SUP precursor-miRNA and derived mature miRNA are newly expressed and are assessed in Section 3.5.2.2.

3.5.1.3 | *Effect of processing*

Maize MON 94804 will undergo existing production processes used for conventional maize. No novel production process is envisaged. Based on the outcome of the comparative assessment, processing of the GM maize into food and feed products is not expected to result in products being different from those of conventional non-GM maize varieties.

3.5.2 | Toxicology assessment

3.5.2.1 | *Testing of newly expressed proteins*

No newly expressed proteins are present in maize MON 94804.

3.5.2.2 | *Testing of new constituents other than newly expressed proteins*

Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, no new constituents have been identified in grain and forage from maize MON 94804, with the exception of the intended expression of GA20ox_SUP precursor-miRNA and derived mature miRNA, designed to reduce plant height by the reduction of the levels of gibberellic acid in the stalk. According to the data provided by the applicant, GA20ox_SUP miRNA down-regulates the expression of the *GA20ox3 and GA20ox5* genes, the products of which are involved in the biosynthetic pathway of gibberellic acid.

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

Moreover, the GMO Panel noted that the GA20ox_SUP miRNA precursor has a typical hairpin structure and does not contain any other structural modification aimed to increase stability or cellular uptake in the gastrointestinal tract following oral administration.

Therefore, it is highly unlikely that the GA20ox_SUP precursor-miRNA and derived mature miRNA can exert biological effects once ingested by humans, mammals, birds and fish. Taking into account all of the above, the GMO Panel considers that no toxicological studies are necessary.

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

No altered levels of food/feed constituents have been identified in maize MON 94804, except for a decrease in gibberellins in several tissues which represents the intended effect of the genetic modification. These changes are considered not to pose a toxicological concern, considering the biological role of the affected constituents and the magnitude of the changes. Therefore, no further toxicological studies are needed.

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

Based on the outcome of molecular characterisation and comparative analysis assessment, no compositional modifications or indication of possible unintended effects relevant to food and feed safety have been identified for maize

[26]Dossier: Part II – Sections 1.4, 1.5, 1.6, 2, 3 and 4; additional information: 10/1/2024.

MON 94804. Therefore, animal studies with food/feed derived from maize MON 94804 are not considered necessary by the GMO Panel (EFSA GMO Panel, 2011a). In accordance with Regulation (EU) No 503/2013, the applicant provided a 90-day feeding study in rats fed with diets containing grains/meal (toasted and defatted) derived from maize MON 94804.

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

Groups were fed diets containing maize MON 94804 grains at 50% or 33% inclusion level (the latter supplemented with 17% of the conventional counterpart) or grains derived from the conventional counterpart (inclusion level 50%).

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

The stability of the test and control materials was not analytically verified; however, it was confirmed that the diet was used in accordance with the product expiration declared by the diet manufacturer. The GMO Panel considered this acceptable evidence that the constituents of the diets would be stable for the duration of the treatment. Furthermore, 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 94804 in both the GM grains and diets and excluded the presence of the event in the respective controls.

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

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

An appropriate range of statistical tests was performed on the results of the study. A detailed description of the methodology and the statistically significant findings identified in rats given diets containing grains derived from maize MON 94804 is reported in Appendix A.

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:

- they were within the normal variation[27] for the parameter in rats of this age;
- they were of small magnitude;
- they were identified at only a small number of time intervals with no impact on the overall value;
- they exhibited no consistent pattern with related parameters or endpoints;
- they exhibited no consistency with increasing incorporation levels.

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

The GMO Panel concludes that this study is in line with the requirements of Regulation (EU) No 503/2013 and that no treatment-related adverse effects were observed in rats after feeding diets containing maize MON 94804 grains at 50% or 33% inclusion level for 90 days.

3.5.3 | Allergenicity

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

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

No newly expressed proteins are present in maize MON 94804.

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

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

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

In the context of this application and considering the information from the molecular characterisation, the compositional analysis and the assessment of the newly expressed constituents in Sections 3.3, 3.4 and 3.5, the GMO Panel identifies no indications of a potentially increased allergenicity of food and feed derived from maize MON 94804 with respect to that derived from the conventional counterpart and the non-GM reference varieties tested.

3.5.4 | Dietary exposure assessment to new constituents

Human and animal dietary exposure assessment to GA20ox_SUP precursor miRNA and derived mature miRNA was not conducted because these molecules are generally rapidly denaturated, depurinated and degraded shortly after ingestion, and therefore, they are considered generally not to exert any biological effects once ingested by humans and animals (see Section 3.5.2.2).

3.5.5 | Nutritional assessment of endogenous constituents

The intended trait of maize MON 94804 is reduced plant height, with no intention to alter nutritional parameters. However, levels of carbohydrates in forage were significantly different from the conventional counterpart and showed a lack of equivalence with the set of non-GM reference varieties (Section 3.4.7).

Animal nutrition

Forage guarantees the correct functioning of the gastrointestinal tract in animals, and represents an important source of feed for herbivores, due to the ability of microbial digestion of the constituents of the carbohydrate plant cell wall, satisfying their nutritional needs up to a certain level, e.g. low producing dairy cows. However, the small decrease in carbohydrate content in MON 94804 maize forage does not represent a nutritional concern.

3.5.6 | Post-market monitoring of GM food/feed

Maize MON 94804, as described in this application, does not raise any nutritional concern and is as safe as its conventional counterpart and the non-GM reference varieties tested. The GMO Panel concludes that, based on the information considered in its safety assessment, a post-market monitoring plan for food and feed is not necessary.

3.5.7 | Conclusions on the food/feed safety assessment

The GA20ox_SUP precursor-miRNA and derived mature miRNA newly expressed in maize MON 94804 do not raise safety concerns for human and animal health. Similarly, the GMO Panel did not identify indications of safety concerns regarding allergenicity or adjuvanticity in maize MON 94804. The GMO Panel finds no evidence that the genetic modification impacts the overall safety of maize MON 94804. Based on the outcome of the comparative assessment and the nutritional assessment, the GMO Panel concludes that the consumption of maize MON 94804 does not represent any nutritional concern, in the context of the scope of this application. The GMO Panel concludes that maize MON 94804, 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.

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

3.6 | Environmental risk assessment and monitoring plan[29]

3.6.1 | Environmental risk assessment

Considering the scope of application GMFF-2022-10651, which excludes cultivation, the environmental risk assessment (ERA) of maize MON 94804 mainly takes into account: (1) the exposure of microorganisms to recombinant DNA in the gastrointestinal tract of animals fed with GM material and of microorganisms present in environments exposed to faecal material of these animals (manure and faeces); and (2) the accidental release into the environment of GM material, including viable maize MON 94804 grains, during transportation and/or processing (EFSA GMO Panel, 2010a).

3.6.1.1 | *Persistence and invasiveness of the GM plant*

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

It is unlikely that the intended trait of maize MON 94804 will provide a selective advantage to maize plants. Therefore, the presence of the intended trait will not affect the persistence and invasiveness of the GM plant.

In conclusion, the GMO Panel considers that maize MON 94804 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 94804 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.

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

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

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

The bioinformatic analysis for event MON 94804 revealed no homology with known DNA sequences from bacteria which would facilitate homologous recombination.

In summary, there is no indication for an increased likelihood of horizontal transfer of DNA from maize MON 94804 to bacteria. Given the nature of the recombinant DNA, the GMO Panel identified no safety concern linked to an unlikely but theoretically possible HGT.

[29]Dossier: Part II – Sections 5 and 6.

Plant to plant gene transfer

The potential for occasional feral maize MON 94804 plants originating from grain import spills to transfer recombinant DNA to sexually compatible plants and the environmental consequences of this transfer were considered.

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

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

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

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

Taking the scope of application GMFF-2022-10651 (no cultivation) and the absence of target organisms into account, potential interactions of occasional feral maize MON 94804 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*

The GMO Panel evaluated the potential hazards of the miRNA and considered that the environmental exposure of non-target organisms to spilled GM maize material or occasional feral GM maize plants arising from spilled maize MON 94804 grains will be limited. Additionally, ingested ncRNAs are typically degraded before entering the environment through faecal material of animals fed with GM maize (Dávalos et al., 2019). Given the limited environmental exposure, the GMO Panel considers that potential interactions of maize MON 94804 with non-target organisms do not raise any environmental safety concern.

3.6.1.5 | *Interactions with abiotic environment and biogeochemical cycles*

The GMO Panel evaluated the potential hazards of the miRNA and considered that the environmental exposure to spilled GM maize material or occasional feral GM maize plants arising from spilled maize MON 94804 grains will be limited. Additionally, ncRNA are typically degraded before entering the environment through faecal material of animals fed with GM maize (Dávalos et al., 2019). Given the limited environmental exposure, the GMO Panel considers that potential interactions of maize MON 94804 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 94804, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for maize MON 94804 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 94804. 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 94804 would differ from conventional maize varieties in its ability to persist under European environmental conditions. Considering the scope of application GMFF-2022-10651, interactions of occasional feral maize MON 94804 plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from maize MON 94804 to bacteria does not indicate a safety concern. Therefore, considering the introduced trait, the outcome of the agronomic and phenotypic analysis, and the routes and levels of exposure, the GMO Panel concludes that maize MON 94804 would not raise safety concerns in the event of accidental release of GM material, including viable GM maize grains, into the environment.

The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of maize MON 94804.

4 | OVERALL CONCLUSIONS

The GMO Panel was asked to carry out a scientific assessment of maize MON 94804 for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003. The molecular characterisation data establish that maize MON 94804 contains a single insert consisting of one copy of the *GA20ox_SUP* suppression cassette. The quality of the sequencing methodology and data sets was assessed by the EFSA GMO Panel and is in compliance with the requirements listed in the EFSA GMO Panel Technical Note. Bioinformatic analyses of the newly created ORFs within the insert or spanning the junctions between the insert and genomic DNA do not raise any safety concerns. The *in planta* RNAi off-target search, performed with the sequence of the GA20ox_SUP miRNA, does not provide indication of an off-target effect that would require further safety assessment. The stability of the inserted DNA and of the introduced trait is confirmed over several generations. 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 differences identified in agronomic, phenotypic and compositional characteristics between maize MON 94804 and its conventional counterpart needed further consideration except for ear height, plant height and levels of carbohydrates in forage, which did not raise concerns when assessed for food/feed and environmental safety. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the GA20ox_SUP precursor-miRNA and derived mature miRNA as expressed in maize MON 94804. The GMO Panel finds no evidence that the genetic modification would change the overall safety of maize MON 94804. In the context of this application, the consumption of food and feed from maize MON 94804 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that maize MON 94804 is as safe as the conventional counterpart and non-GM maize varieties tested, and no post-market monitoring of food/feed is considered necessary. The GMO Panel concludes that there is a very low likelihood of environmental effects resulting from the accidental release of viable grains from maize MON 94804 into the environment. The PMEM plan and reporting intervals are in line with the intended uses of maize MON 94804. Based on the results of the literature searches, the GMO Panel did not identify any safety issues pertaining to the intended uses of maize MON 94804. The GMO Panel concludes that maize MON 94804 is as safe as its conventional counterpart and the tested non-GM maize reference varieties with respect to potential effects on human and animal health and the environment.

5 | DOCUMENTATION AS PROVIDED TO EFSA

- Letter from the Competent Authority of The Netherlands received on 14 February 2023 concerning a request for authorization of the placing on the market of genetically modified maize MON 94804 submitted in accordance with Regulation (EC) No 1829/2003 by Bayer Agriculture (EFSA Ref. EFSA-GMFF-2022-10651; EFSA-Q-2023-00106).
- The application was made valid on 2 May 2024.
- Additional information (1) was requested on 7 June 2023.
- Additional information (1) was received on 16 January 2020.
- Additional information (2) was requested on 16 October 2023.
- Additional information (2) was received on 16 October 2023.
- Additional information (3) was requested on 14 November 2023.
- Additional information (3) was received on 10 January 2024.
- Additional information (4) was requested on 21 December 2023.
- Additional information (4) was received on 10 January 2024.
- Additional information (5) was requested on 16 January 2024.
- Additional information (5) was received on 8 February 2024.

ABBREVIATIONS

CRM comparative relative maturity
dw dry weight
ERA environmental risk assessment
FOB functional observational battery
GA20ox gibberellic acid 20 oxidase
GLP good laboratory practice
GMO genetically modified organism
HGT horizontal gene transfer
HR homologous recombination
JSA Junction Sequence Analysis
miRNA microRNA
ncRNA non-coding RNA
NGS Next Generation Sequencing
OECD Organisation for Economic Co-operation and Development
ORFs open reading frames
OSL over season leaf
PCR polymerase chain reaction
PMEM post-market environmental monitoring
T-DNA transfer-deoxyribonucleic acid
UTR untranslated region

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CONFLICT OF INTEREST

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

REQUESTOR

Competent Authority of The Netherlands.

QUESTION NUMBER

EFSA-Q-2023-00106

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SUPPORTING INFORMATION

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

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APPENDIX A

Statistical analysis and statistically significant findings in the 90-day toxicity study in rats on maize MON 94804

A.1 STATISTICAL ANALYSIS OF THE 90-DAY STUDY ON MAIZE MON 94804 IN RATS

TABLE A.1 Statistically significant findings in the 90-day study on maize MON 94804 in rats.

Statistically significant parameter/endpoint	Finding (versus control)	GMO panel interpretation
Body weight gain	Increased 9% in high dose group (sexes combined), weeks 0–6 & 0–7.	Low magnitude. Terminal body weights within 5% of controls. Within normal variation. Not an adverse effect of treatment.
Food consumption	Increased 9% in high dose group (sexes combined), weeks 2–3 & 12–13.	Low magnitude. Not seen consistently. Within normal variation. Not an adverse effect of treatment.
Urine pH	Increased in low dose animals (sexes combined).	Low magnitude. Not seen in the high dose animals. All values within normal variation/the control range. Not an adverse effect of treatment.
TSH	Increased (55%) in high dose group (sexes combined); in females over 3 times higher.	Appears to be driven by unusually low TSH values in female controls; high dose values are consistent with the normal background values. No indication of perturbed thyroid homeostasis. No related changes in thyroid weights, T3 or T4 levels. No adverse pathology findings in the thyroid. Not an adverse effect of treatment.

The following endpoints were statistically analysed: body weight, body weight changes, food consumption, clinical pathology values, absolute and relative organ weights, functional observational battery (FOB) data, locomotor activity and histopathological data. For all continuous endpoints, mean and standard deviation were provided for each dose group and for each sex, variable and period or time interval. In the statistical analysis, rats consuming the low-and high-dose test diets were compared with those consuming the control diet. For continuous parameters, a linear mixed model was applied to data for individual animals for the two sexes combined (fixed effects: diet, sex and sex-by-diet interaction; random effects: block-within-sex and the interaction of block and dose within sex, the latter representing the cage effect). The model was modified as needed for the analysis of sex-specific endpoints, of endpoints with cage-level data (food consumption and food efficiency) and of continuous locomotor activity endpoints (by including time interval as an additional fixed effect). For all the models, in case the sex-by-treatment interaction was significant, the comparison was done separately for males and females. For each comparison, point estimates and 95% confidence intervals of the standardised effect size were reported to aid the assessment. For categorical FOB parameters, the comparisons were performed with Fisher's exact test separately for each sex and period. Missing data were considered by the GMO Panel and found not to affect the results.[30]

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