Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on application GMFF-2022-10651 (maize MON 94804) from Bayer under Regulation (EC) No. 1829/2003

11 June 2024
Ref. SC/1510/BAC/2024_0798

Context

Application GMFF-2022-10651 was submitted by Bayer for the authorisation for the marketing of genetically modified (GM) maize MON 94804 (Unique Identifier MON-948Ø4-4) for food and feed uses, import and processing (excluding cultivation) within the European Union, within the framework of Regulation (EC) No. 1829/2003[1].

MON 94804 contains a single insert consisting of one copy of the *GA20ox* suppression cassette, expressing a miRNA suppressing the expression of maize endogenous gibberellic acid 20 oxidase genes *ZmGA20ox3* and *ZmGA20ox5* (hereafter referred to as GA20ox_SUP miRNA). The reduction of gibberellic acid in the stalk, leads to reduced plant height. MON 94100 is going to be used to produce stacked events via conventional breeding and will not be commercialised as a stand-alone product. The assessment and opinion by the Belgian Biosafety Advisory Council (BAC) presented below are therefore for a hypothetical product.

The application was validated by EFSA on 2 May 2023 and a formal three-month consultation period of the Member States was started, lasting until 3 August 2023, in accordance with Articles 6.4 and 18.4 of Regulation (EC) No. 1829/2003 (consultation of national Competent Authorities within the meaning of Directive 2001/18/EC designated by each Member State in the case of genetically modified organisms being part of the products).

Within the framework of this consultation, the Belgian Biosafety Advisory Council (BAC), under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier, chosen from the common list of experts drawn up by the BAC and the Service Biosafety and Biotechnology (SBB). Four experts answered positively to this request, and formulated a number of comments to the dossier. See Annex I for an overview of all the comments and the comments sent to EFSA on 13 July 2023.

The opinion of the EFSA Scientific Panel on GMOs was published on 26 April 2024 (EFSA Journal 2024;22:e8714[2]) together with the responses from the EFSA GMO Panel to comments submitted by the Member States during the three-month consultation period. Those documents were forwarded to the experts on 6 May 2024, with an invitation to react if needed.

In delivering the present advice, the BAC considered in particular the comments formulated by the experts on application GMFF-2022-10651 and the opinion of EFSA.

[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).
[2] See: https://doi.org/10.2903/j.efsa.2024.8714

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Scientific evaluation

1. Molecular characterisation

With regard to the molecular characterisation, it was noted that the GA20ox-SUP mRNAi is not only expressed in the stalk, but also in other maize tissues. As no unexpected changes were observed in the agronomic and phenotypic endpoints, the Biosafety Advisory Council is of the opinion that the information provided is sufficient and does not raise safety concerns.

2. Assessment of food/feed safety and nutritional value

2.1. Assessment of compositional analysis

The Biosafety Advisory Council agrees with the GMO panel of EFSA that the compositional data of GM maize MON 94804, in comparison with its conventional counterpart, do not raise safety concerns.

2.2. Assessment of toxicity

Given the nature of the new constituent GA20ox_SUP miRNA (i.e. it is a non-coding RNA with a typical hairpin structure that is by nature ubiquitous in food/feed), the Biosafety Advisory Council is of the opinion that there are no safety concerns with respect to toxicity.

2.3. Assessment of allergenicity

Given the nature of the new constituent GA20ox_SUP miRNA (i.e. it is a non-coding RNA with a typical hairpin structure that is by nature ubiquitous in food/feed), the Biosafety Advisory Council is of the opinion that there are no safety concerns with respect to allergenicity.

2.4. Nutritional value

The Biosafety Advisory Council is of the opinion that the information provided is sufficient to conclude that the nutritional characteristics of maize MON 94804-derived food and feed are not expected to differ from those of conventional maize varieties.

3. Environmental risk assessment

Field observations indicate that maize grains can sometimes overwinter and germinate in certain regions of the EU (e.g. Palaudelmàs *et al*., 2009[3]; COGEM, 2011[4]; Pascher, 2016[5]). As a result, volunteer maize plants do sometimes occur in subsequent crops. There is also evidence of the rare occurrence of feral maize plants (e.g. Pascher, 2016; COGEM, 2018[6]). However, volunteer maize has been shown to grow weakly and is not considered an agricultural problem. There are no indications that the occurrence of feral maize plants has resulted in the establishment of self-sustaining populations. This can be explained by the fact that maize is highly domesticated, has no weedy characteristics and is not tolerant to frost. Thus, the occurrence of volunteer and feral maize in the EU is currently limited and transient. In addition, maize has no sexual compatible wild relative in the EU. Therefore, the Biosafety Advisory Council is of

[3] Palaudelmàs M., *et al*., 2009. Effect of volunteers on maize gene flow. Transgenic Res.18(4):583-594. doi:10.1007/s11248-009-9250-7

[4] COGEM, 2011. Research report "Crop volunteers and climate change. Effects of future climate change on the occurrence of maize, sugar beet and potato volunteers in the Netherlands". https://cogem.net/en/publication/crop-volunteers-and-climate-change-effects-of-future-climate-change-on-the-occurrence-of-maize-sugar-beet-and-potato-volunteers-in-the-netherlands/

[5] Pascher K., 2016. Spread of volunteer and feral maize plants in Central Europe: recent data from Austria. Environ. Sci Eur.28(1):30. doi:10.1186/s12302-016-0098-1

[6] COGEM, 2018. Research report "Are teosinte and feral maize present in the Netherlands?". https://cogem.net/en/publication/are-teosinte-and-feral-maize-present-in-the-netherlands/

the opinion that it is unlikely that the accidental release of maize MON 94804 (i.e. during transport and/or processing) into the European environment[7] will lead to environmental harm.

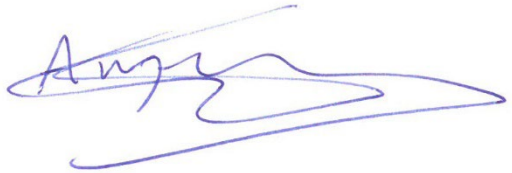
4. Monitoring

With regard to monitoring, the Biosafety Advisory Council is of the opinion that the information provided is sufficient.

Conclusion

Based on the whole set of data on maize MON 94804 provided by the applicant, the scientific assessment of the dossier done by the Belgian experts, the scientific opinion of EFSA, and the answers of the EFSA GMO panel to the questions raised by the Belgian experts, the Biosafety Advisory Council:

1) Agrees with the GMO panel of EFSA that maize MON 94804 would not raise safety concerns in the case of accidental release of viable GM maize grains into the environment;
2) Agrees with the GMO panel of EFSA that in the context of its proposed uses, maize MON 94804 is as safe as its conventional counterpart and the tested non-GM reference varieties with respect to potential effects on human and animal health.



Dr. ir. Geert Angenon
President of the Belgian Biosafety Advisory Council

Annex : Outcome of the assessment of the application and comments sent to EFSA

[7] As the application doesn't imply cultivation of the GM crop in the EU, a full environmental assessment, as in the case of a cultivation dossier, is not warranted.

Annex : Outcome of the assessment of application GMFF-2022-10651 by the Biosafety Advisory Council during the formal consultation of the Member States (3-month commenting period in accordance with Articles 6.4 and 18.4 of Regulation (EC) No 1829/2003) and feedback from the EFSA GMO Panel

Coordinator: Lieve Gheysen (UGent)
Experts: Eddy Decuypere (KUL), Patrick du Jardin (ULg), André Huyghebaert (UGent), Peter Smet (consultant)
SBB: Adinda De Schrijver

Application: **GMFF-2022-10651**
Applicant: **Bayer CropScience**
GMO: **maize MON 94804**
Validation of dossier by EFSA: **2 May 2023**

Scope of the application:
☒ GM plants for food use
☒ Food containing or consisting of GM plants
☒ Food produced from GM plants or containing ingredients produced from GM plants
☒ GM plants for feed use
☒ Feed produced from GM plants
☒ Import and processing (Part C of Directive 2001/18/EC)
☐ Seeds and plant propagating material for cultivation in European Union (Part C of Directive 2001/18/EC)

Given the characteristics of the GMO and its intended uses, experts were consulted to cover the following areas of expertise:
☒ Molecular characterization
☒ Environmental aspects
☒ Allergenicity
☒ Toxicology
☒ Food and Feed aspects

The experts were asked to evaluate whether the information provided in the application is sufficient in order to state that the marketing of the genetically modified plant for its intended uses, will not raise any problems for the environment or human or animal health. If information is lacking, the expert was asked to indicate which information should be provided and what the scientifically reasoning is behind this demand.

Annex I provides an overview of risk assessment related comments received that fall within the remit of the Biosafety Advisory Council. Comments sent to EFSA are highlighted in grey, with the answers from the GMO Panel from EFSA provided underneath. It should be noted that all the comments mentioned

in Annex I were considered in the evaluation of this dossier and in formulating the final advice of the Biosafety Advisory Council.

Annex II provides an overview of other comments received that do not fall within the remit of the work of the Biosafety Advisory Council, such as comments related to the plant protection product used on the GM plant and Maximum Residue Levels of herbicides, and statements on GMOs (e.g. socio-economic considerations) or statements without supporting reasoning or evidence.

Annex I - List of risk assessment related comments/questions received from the experts

PART II - SCIENTIFIC INFORMATION

1. HAZARD IDENTIFICATION AND CHARACTERISATION

1.1. INFORMATION RELATING TO THE RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANTS

Have evaluated this section and consider the information adequate: 1 expert

Comment:
The possible cross-pollination is discussed in section 1.1.5.2 where it is stated "The amount of pollen-mediated gene flow is greatest within the first few meters and decreases sharply with increasing distance...". This is probably underestimated. Some of the references cited by the authors themselves speak of more than a few tens of meters before the flow decreases. Other simulations (Hu et al. 2022) even consider more than a few hundred meters. Furthermore, it is also questionable whether 0.1% gene flow at a certain distance is satisfactory; this flow is not negligible.

A large scale study of Fernandes et al. (2022), in Brazil, shows also gene flow between GM and non-GM plants even if the distances legally fixed are respected.

Note SBB & coordinator: While gene flow from maize has been reported at greater distances up to a few hundred meters, it is indeed generally recognised that most of the pollen is deposited within about 30 m from the source (e.g. Devos et al., 2005, Environ. Biosafety Res. 4, 71-78).

1.2. MOLECULAR CHARACTERISATION

1.2.1. Information relating to the genetic modification

Comment 1:
The GA20ox_SUP miRNA insert in MON 94804 after expression induces the generation of small RNAs that target the transcripts of the ZmGA20ox3 and ZmGA20ox5 gibberellic acid 20 oxidase (GA20ox) genes but not those of the related ZmGA20ox1 gene. The comparative analysis of the transcripts of these 3 genes in different tissues presented among others in Tables 1-3 (Study# REG - 2021-0195) shows unexpected results which are little commented on or quickly evacuated. In particular, although GA20ox_SUP miRNA is not detectable by Northern blot in pollen grains (and seeds), there are statistically ($p<0.0001$) more ZmGA20ox3 transcripts in MON 94804 pollen

grains than in the conventional control (but not in the seeds). Knowing that gibberellins regulate the viability, germination, and growth of the pollen tube (Plant Cell. 19: 3876–3888) it would be informative to conduct a comparative analysis of germination (as done with seeds) and growth of the pollen tube to complete the phenotypic analysis of MON 94804. In general, I note that the relative expression of the GA20ox genes/gibberellins content in the different tissues of MON 94804 is not sufficiently discussed, and this in relation to the phenotypic observations.

Note SBB & coordinator: While the information on the germination of pollen can further establish if MON 94804 is phenotypically equivalent to conventional maize, we doubt it will aid in the evaluation of the risks for human health and the environment. GA can indeed stimulate pollen tube growth and pollen viability, but even if pollination would be a little more efficient, we do not see how this could be harmful to the environment. It could be asked to comment on this but we do not see the need for extra experiments.

Feedback from the EFSA GMO Panel: The GMO Panel thanks Belgium for the comment. The GMO Panel considers that the suppression of GA20ox genes produced the intended effect and no unexpected changes were observed in the agronomic and phenotypic endpoints (see Section 3.4.5 of the Scientific Opinion). In addition, the GMO Panel did not find any indications of an off-target effect of the GA20ox SUP miRNA expression that would need further safety assessment.

Comment 2:
Section 1.2.1.3(b): I would like to temper the statements “There is a history of safe consumption of the RNA molecules mediating gene suppression in plants (…)", which is supported by citing papers of Jensen et al. (2013) and Ivashuta et al. (2009) (both Monsanto publications). Note that the Papadopulou et al. (2020) EFSA paper is also cited below.

At present, there is at least one publication that seemed to show an influence on gene expression following miRNA ingestion (Zhang et al. 2012), but this publication is clearly controversial. However, it should be borne in mind that the debate is not over (Nawaz et al. 2019).

The Papadopoulou et al. (2020) and Davalos et al. (2019) papers indicate that the results cited above (Zhang et al 2012) could be due to technical artefacts or contamination, but Davalos et al. (2019) also indicates that further research is needed.

Note coordinator: I agree with the applicant that there is no real evidence that plant RNAs can have negative effects on the human body, however, to state that there is a history of safe consumption might be a bit too strong.

1.2.2. Information relating to the genetically modified plant

Have evaluated this section and consider the information adequate: 1 expert

Comment:
All the bioinformatics analyses concerning possible off targets seem sound to me, and I agree with the results.

However, the papers by Papadopoulou et al (2020) and Davalos et al (2019) show that chemical modifications to ncRNAs (non-coding RNAs) expressed in plants can increase their stability (both inside the plant cell and outside the plant). I did not see any analysis of this aspect in the application submitted. In my opinion, this aspect should be addressed.

Note SBB: From our understanding of the information in the dossier no chemical modifications were done to increase the stability of GA20ox_SUP miRNA.

1.2.3. Additional information relating to the genetically modified plant required for the environmental safety aspects

Have evaluated this section and consider the information adequate: 2 experts

1.2.4. Conclusions of the molecular characterisation

Have evaluated this section and consider the information adequate: 2 experts

1.3. COMPARATIVE ANALYSIS

1.3.1. Choice of the conventional counterpart and additional comparators

Have evaluated this section and consider the information adequate: 1 expert

Comment:

The approach is in line with previous applications.

1.3.2. Experimental design and statistical analysis of data from field trials for comparative analysis

Have evaluated this section and consider the information adequate: 2 experts

1.3.3. Selection of material and compounds for analysis

Have evaluated this section and consider the information adequate: 1 expert

Comment:

The OECD document of 2002 on compositional considerations of new varieties of maize, was followed. This document needs an update in order to include recent insights in the nutritional value of foods.

1.3.4. Comparative analysis of composition

Have evaluated this section and consider the information adequate: 1 expert

Comment:

The EFSA guidelines were used for the statistical analysis of the data.

1.3.5. Comparative analysis of agronomic and phenotypic characteristics

Have evaluated this section and consider the information adequate: 1 expert

1.3.6. Effects of processing

Comment:

Taking into account the compositional equivalence of maize MON 94804 with reference maize and commercial varieties, it can expected that there is no effect on the processing of maize by dry and wet milling.

1.3.7. Conclusion

Have evaluated this section and consider the information adequate: 1 expert

Comment:
The applicant concludes from the compositional and agronomic characteristics, that there is no need for a further assessment of food and feed safety aspects. I agree with this conclusion.

1.4. TOXICOLOGY

1.4.1. Testing of newly expressed proteins

Have evaluated this section and consider the information adequate: 1 expert

Comment:
The GA20ox SUP suppression cassette does not code for any protein. Therefore, this section is not applicable.

1.4.2. Testing of new constituents other than proteins

Have evaluated this section and consider the information adequate: 1 expert

Comment:
Based on the information and references provided, the expressed GA20ox SUP miRNA is considered to pose negligible risk to human and animal health.

1.4.3. Information on natural food and feed constituents

Have evaluated this section and consider the information adequate: 1 expert

Comment:
As described in Section 1.3, no relevant changes in the composition of MON 94804 were detected compared to its conventional counterpart. Therefore, the levels of food and feed constituents in MON 94804 have not been altered.

1.4.4. Testing of the whole genetically modified food or feed

Have evaluated this section and consider the information adequate: 1 expert

Comment:
The report M-827778-01-1 2023 of the 90-day feeding study with diet containing maize grain from MON 94804 was reviewed. The review confirmed the conclusion that dietary administration of maize grain from MON 94804 at a concentration of 33% (w/w) and 50% (w/w) in the diet for at least 90 consecutive days had no adverse effects on the growth or health of Sprague Dawley (Crl:CD[SD]) rats. There were no findings which triggered the need for additional safety studies.

1.4.5. Conclusion of the toxicological assessment

Have evaluated this section and consider the information adequate: 1 expert

Comment:
The provided information indicated that it is highly unlikely that MON 94804 would cause any adverse effects on human or animal health. There was no finding triggering the need for additional safety studies.

1.5. ALLERGENICITY

1.5.1. Assessment of allergenicity of the newly expressed protein

Have evaluated this section and consider the information adequate: 2 experts

1.5.2. Assessment of allergenicity of the whole genetically modified plant

Have evaluated this section and consider the information adequate: 2 experts

1.5.3. Adjuvanticity

Have evaluated this section and consider the information adequate: 1 expert

1.5.4. Conclusion of the allergenicity assessment

Have evaluated this section and consider the information adequate: 2 experts

1.6. NUTRITIONAL ASSESSMENT

1.6.1. Nutritional assessment of the genetically modified food

Have evaluated this section and consider the information adequate: 1 expert

1.6.2. Nutritional assessment of the genetically modified feed

Have evaluated this section and consider the information adequate: 1 expert

1.6.3. Conclusion of the nutritional assessment

Have evaluated this section and consider the information adequate: 1 expert

2. EXPOSURE ASSESSMENT — ANTICIPATED INTAKE OR EXTENT OF USE

No feedback received

3. RISK CHARACTERISATION

Have evaluated this section and consider the information adequate: 2 experts

4. POST-MARKET MONITORING ON THE GENETICALLY MODIFIED FOOD OR FEED

No feedback received

5. ENVIRONMENTAL RISK ASSESSMENT (ERA)

5.1. INTRODUCTION

Have evaluated this section and consider the information adequate: 2 experts

5.2. GENERAL APPROACH OF THE ERA

Have evaluated this section and consider the information adequate: 2 experts

5.3. SPECIFIC AREAS OF RISK

5.3.1. Persistence and invasiveness including plant-to-plant gene flow

Have evaluated this section and consider the information adequate: 2 experts

5.3.2. Plant to micro-organisms gene transfer

Have evaluated this section and consider the information adequate: 2 experts

5.3.3. Interactions of the GM plant with target organisms

Have evaluated this section and consider the information adequate: 2 experts

5.3.4. Interactions of the GM plant with non-target organisms (NTOs)

Have evaluated this section and consider the information adequate: 1 expert

5.3.5. Impacts of the specific cultivation, management and harvesting techniques

No feedback received

5.3.6. Effects on biogeochemical processes

No feedback received

5.3.7. Effects on human and animal health

No feedback received

5.3.8. Overall risk evaluation and conclusions

Have evaluated this section and consider the information adequate: 1 expert

6. POST-MARKET ENVIRONMENTAL MONITORING PLAN (PMEM)

No feedback received

7. ADDITIONAL INFORMATION RELATED TO THE SAFETY OF THE GENETICALLY MODIFIED FOOD OR FEED

No feedback received

8. REFERENCES

- Dávalos et al. Literature review of baseline information on non-coding RNA (ncRNA) to support the risk assessment of ncRNA- based genetically modified plants for food and feed. EFSA Supp. Publ 2019, 16:220.
- Fernandes et al. Transgene Flow: Challenges to the On-Farm Conservation of Maize Landraces in the Brazilian Semi-Arid Region. Plants (Basel) 2022, 11(5):603.
- Hu et al. Application of the maximum threshold distances to reduce gene flow frequency in the coexistence between genetically modified (GM) and non-GM maize. Evol Appl. 2022, 15:471-483

- Jensen et al. Computational sequence analysis of predicted long dsRNA transcriptomes of major crops reveals sequence complementarity with human genes. GM Crops Food. 2013, 4:90-97.
- Ivashuta et al. Endogenous small RNAs in grain: semi-quantification and sequence homology to human and animal genes. Food Chem Toxicol. 2009, 47:353-360.
- Nawaz et al.. Addressing concerns over the fate of DNA derived from genetically modified food in the human body: A review. Food Chem Toxicol. 2019, 124:423-430.
- Papadopoulou et al. Risk Assessment Considerations for Genetically Modified RNAi Plants: EFSA's Activities and Perspective. Front Plant Sci. 2020, 11:445.
- Zhang et al. Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA. Cell Res. 2012, 22:107-26.

Annex II - List of other comments/questions received from the experts

PART II - SCIENTIFIC INFORMATION

1.3.4. Comparative analysis of composition

Comment:

In the document it is mentioned that the practices include monitoring of the fields for insects, diseases and weeds. I found no results on these particular aspects particularly with reference to mycotoxins. Maize is quite sensitive to mycotoxins.

Is it possible under the wide range of conditions of the field trials that there is no mould (mycotoxin) problem? It is clear that there is no indication that maize MON 94804 would be more sensitive to the mycotoxin problem but any data would confirm this statement.