Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on application EFSA-GMO-NL-2019-161 (maize MON 87429) from Bayer under Regulation (EC) No. 1829/2003

30 January 2023
Ref. SC/1510/BAC/2023_0101

Context

Application EFSA-GMO-NL-2019-161 was submitted by Bayer Agriculture BV for marketing authorisation of genetically modified (GM) maize MON 87429 (Unique Identifier MON-87429-9) for food and feed uses, import and processing (excluding cultivation) within the European Union, within the framework of Regulation (EC) No. 1829/2003[1].

Maize MON 87429 contains a single insert containing *dmo, pat, ft_t* and *epsps* expression cassettes to confer tolerance to dicamba-, glufosinate-, 2,4D- and quizalofop, and glyphosate-based herbicides, respectively. MON 87429 also utilises an endogenous maize RNAi regulatory element to suppress *epsps* expression in pollen. Application of glyphosate in the pollen stage will lead to male sterility which will facilitate hybrid seed production.

The application was validated by EFSA on 16 January 2020 and a formal three-month consultation period of the Member States was started, lasting until 17 April 2020, 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 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). Three 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.

The opinion of the EFSA Scientific Panel on GMOs was published on 18 November 2022 (EFSA Journal 2022;20(11):7589[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 15 December 2022, 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 EFSA-GMO-NL-2019-161 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.2022.7589

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Scientific evaluation

1. Molecular characterisation

With regard to the molecular characterisation, 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 87429, in comparison with its conventional counterpart, do not raise safety concerns.

2.2. Assessment of toxicity

The Biosafety Advisory Council has evaluated the safety of the PAT and EPSPS proteins in the context of previous applications and no safety concerns with respect to toxicity were identified. Taking into account the new information in the current application, the Council is of the opinion that the previous conclusions remain valid.
The Biosafety Advisory Council evaluated the safety of the newly produced DMO (dicamba mono-oxygenase) and Ft_t proteins and no safety concerns with respect to toxicity were identified. The DMO protein in MON 87429 differs slightly from DMO proteins present in GM events assessed in the past.
The Biosafety Advisory Council is also of the opinion that the combined presence of the newly expressed proteins in MON 87429 does not raise toxicological concerns.
Further, the Biosafety Advisory Council agrees with the GMO panel of EFSA that the available data on the toxicity of GM maize MON 87429, in comparison with its conventional counterpart, does not raise safety concerns.

2.3. Assessment of allergenicity

The Biosafety Advisory Council has evaluated the safety of the PAT and EPSPS proteins in the context of a previous applications and no concerns were identified with respect to their allergenicity. Taking into account the new information in the current application, the Council is of the opinion that the previous conclusions remain valid.

The Biosafety Advisory Council evaluated the safety of the newly produced DMO (dicamba mono-oxygenase) and Ft_t proteins and no safety concerns with respect to allergenicity were identified.

The Biosafety Advisory Council is also of the opinion that the combined presence of the newly expressed proteins in MON 87429 does not raise concerns regarding 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 87429-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. The occurrence of feral maize plants has not resulted in the establishment of self-sustaining populations, mainly because 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 the opinion that it is unlikely that the accidental release of maize MON 89034 x 1507 x MIR162 x NK603 x DAS-40278-9 (i.e. during transport and/or processing) into the European environment[7] will lead to environmental harm.

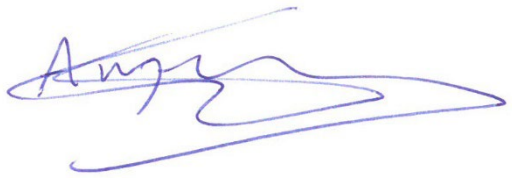
4. Monitoring

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 87429 provided by the applicant, the scientific assessment of the dossier done by the Belgian experts, and the opinion of EFSA, the Biosafety Advisory Council:

1) Agrees with the GMO panel of EFSA that the potential environmental release of maize MON 87429 is unlikely to pose any threat to the European environment;
2) Agrees with the GMO panel of EFSA that maize MON 87429 is as safe as the conventional counterpart and non-GM maize reference varieties.



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

Annex: Outcome of the assessment of the application EFSA-GMO-NL-2019-161.

[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/
[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.

Minority declaration of P. Baret

On point 3 of this advice (Environmental risk assessment), the presence of feral populations of maize in Europe is mentioned but no proper assessment of the risk related to these populations is proposed. As the maize is resistant to herbicides, the potential impact on biodiversity and on coexistence implies scientific data on fitness and survival. In absence of a comprehensive science based risk assessment, it is impossible to conclude that there is no environmental risk.

Annex: Outcome of the assessment of application EFSA/GMO/NL/2019/161 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 EFSA GMO Panel

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Experts: Lieve Gheysen (UGent), André Huyghebaert (UGent), Peter Smet (Consultant)
SBB: Adinda De Schrijver

Application: EFSA/GMO/NL/2019/161
Applicant: Bayer Agriculture BV
GMO: maize MON 87429
Validation of dossier by EFSA: 16 January 2020

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.

None of the comments provided by the experts were sent to EFSA. It should be noted that all the comments received from the experts are considered in the evaluation of this dossier and in formulating the final advice of the Biosafety Advisory Council.

List of comments/questions received from the experts

PART I - GENERAL INFORMATION

No comments received

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

1.2. MOLECULAR CHARACTERISATION

1.2.1. Information relating to the genetic modification

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

1.2.2. Information relating to the genetically modified plant

Comment:

Sequence analysis was used to exclude the presence of vector backbone DNA in MON 87429, with appropriate control (spiking with plasmid down to 1/10 genome equivalents). The applicants claim (p43) that any inserted DNA should come from the plasmid PV-ZMHT519224. This is not entirely correct. Sequences corresponding to chromosomal *Agrobacterium* DNA have been detected in transgenic plants (Ülker et al., 2008). However, they are generally inserted linked to the T-DNA and should therefore have been detected in the sequence analysis. This was not the case as the T-DNA was found to be linked to plant DNA at the junctions (Robinson et al., 2019, ref in dossier). In conclusion, there is no chromosomal *Agrobacterium* DNA in MON 87429.

On p44, the applicants say “the presence of two junctions indicate a single intended T-DNA at a single locus in the genome of MON 87429”. This is true unless there are two tandem copies of the T-DNA or three copies in inverted repeats. In the case of two tandem copies, a junction of right and left border sequences should have been detected, which is not reported. Three copies in inverted repeats are not easy to detect by sequence analysis and certainly not by PCR as inverted repeats are not well amplified. However, it is rather unlikely that MON 87429 contains inverted T-DNA repeats as this would have a high chance of causing gene silencing.

Nevertheless, I wonder if the sequence analysis performed would be able to distinguish -by the number of genomic sequence reads- if MON 87429 could have more than 1 T-DNA copy.

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: 1 expert

1.2.4. Conclusions of the molecular characterisation

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

1.3. COMPARATIVE ANALYSIS

1.3.1. Choice of the conventional counterpart and additional comparators

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

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: 1 expert

1.3.3. Selection of material and compounds for analysis

Comment:
A conventional counterpart was chosen with background genetics similar to maize 161.

1.3.4. Comparative analysis of composition

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

Comment:
The assessment is based upon analysis of constituents according to the OECD guidelines of 2002. Attention is given to proximate analysis, nutrients, anti-nutrients and secondary metabolites in grain. Results for forage are not included in my comments.

I paid some particular attention to the nutrient levels of maize grain, not treated.

Protein and amino acids.
One of the 19 amino acids, methionine, was found to be significant different. Values are however within the range of conventional counterpart.

Total fat and fatty acids
Four of the 10 components were found to be not significant different. Total fat was significantly different, with a difference of - 0.34 %. For the other fatty acids, found to be significantly different, the difference was 0.0066 % and 0.25 %. The magnitude of the difference was not considered meaningful. In addition, the values overlapped with the range of values of the conventional counterpart.

No data are given for the phytosterols. Maize germ oil is one of the richest sources of phytosterols between vegetable oils. As phytosterols affect cholesterol metabolism in a positive way, some more attention would be welcome.

Carbohydrates, moisture and fibre.
As mentioned before in precious reviews of similar applications, I have some comments on the methods used in this chapter. Carbohydrates, assessed by calculation is not accepted anymore as a valuable approach. The same applies for the fibre results. I understand that the OECD document accepted these methods in 2002. Today this approach is no longer accepted in human nutrition.

On the other hand, I agree with the conclusion of compositional equivalence for carbohydrates.

Ash and mineral
No comment

Vitamins
No statistical difference for vitamin B1, B3, B6 and B9. The applicant concludes that the differences for vitamin A, B2 and E are not meaningful.

Results in table 6 show that the difference for vitamin E between test and conventional counterpart is – 0.89 % mean value and – 7.490 % relative value.
In my opinion a relative value of this order needs further consideration. Maize germ oil is a highly unsaturated oil. Vitamin E is an important vitamin in this oil.

These results apply for not treated maize grain. Results of maize grain treated also show significant differences in α-tocopherol content.

I found no data on the level of different tocopherols. Only α-tocopherol or vitamin E is given. There is no result for the main tocopherols, γ-tocopherol. This compound is important in term of oxidation stability.
No results are given for tocotrienols also important antioxidants

Anti-nutrients and secondary metabolites
no comment

Conclusion
The applicant concludes that based upon the statistical analysis maize 161 is equivalent to the conventional counterpart.
I basically agree with this conclusion. However, I would like to have some information in relation to vitamin E.
I regret that no attention was paid to tocotrienols and to phytosterols.

1.3.5. Comparative analysis of agronomic and phenotypic characteristics

1.3.6. Effects of processing

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

1.3.7. Conclusion

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

Comment:
I agree with the conclusion of compositional equivalence.
I would like to have some more explanation on the results of vitamin E.
I regret that no information is available on the level of phytosterols and tocotrienols.
I repeat my remark on the methods used for the assessment of carbohydrates and fibre.

Note coordinator & SBB: The components analysed by applicants are based on the crop documents of the OECD. Tocotrienols and phytosterols are not included in the list of components to be analysed for maize according to the OECD (see: https://www.oecd.org/env/ehs/biotrack/46815196.pdf). As the results for vitamin E fall within the range of those for the reference varieties, we do not consider this points to a safety issue.

1.4. TOXICOLOGY

1.4.1. Testing of newly expressed proteins

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

1.4.2. Testing of new constituents other than proteins

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

1.4.3. Information on natural food and feed constituents

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

1.4.4. Testing of the whole genetically modified food or feed

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

1.4.5. Conclusion of the toxicological assessment

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

1.5. ALLERGENICITY

No comments received

1.6. NUTRITIONAL ASSESSMENT

No comments received

2. EXPOSURE ASSESSMENT — ANTICIPATED INTAKE OR EXTENT OF USE

No comments received

3. RISK CHARACTERISATION

No comments received

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

No comments received

5. ENVIRONMENTAL RISK ASSESSMENT (ERA)

No comments received

6. POST-MARKET ENVIRONMENTAL MONITORING PLAN (PMEM)

No comments received

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

No comments received

REFERENCES

Ülker, B., Li, Y., Rosso, M. *et al.* T-DNA–mediated transfer of *Agrobacterium tumefaciens* chromosomal DNA into plants. *Nat Biotechnol.* **26,** 1015–1017 (2008). https://doi.org/10.1038/nbt.1491