Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on application EFSA-GMO-DE-2018-149 (genetically modified maize Bt11 x MIR162 x MIR604 x MON 89034 x 5307 x GA21) from Syngenta under Regulation (EC) No. 1829/2003

14 September 2023
Ref. SC/1510/BAC/2023_0904

Context

Application EFSA-GMO-DE-2018-149 was submitted by Syngenta Crop Protection N/SA for the marketing of genetically modified (GM) maize Bt11 x MIR162 x MIR604 x MON 89034 x 5307 x GA21 (Unique Identifier SYN-BT∅11-1 x SYN-IR162-4 x SYN-IR6∅4-5 x MON-89∅34-3 x SYN-∅53∅7-1 x MON-∅∅∅21-9) for food and feed uses, import and processing (excluding cultivation) within the European Union, within the framework of Regulation (EC) No. 1829/2003[1].

The six-event stack, maize Bt11 x MIR162 x MIR604 x MON 89034 x 5307 x GA21, was obtained by conventional crossing (no new genetic modification involved) of the corresponding single events:

- Bt11, expressing the *cry1Ab* gene that confers resistance to certain lepidopteran pests and the *pat* gene that confers tolerance to herbicide products containing glufosinate ammonium;
- MIR162, expressing the *vip3Aa20* gene that confers resistance to certain lepidopteran pests and the *pmi* gene that is used as selectable marker;
- MIR604, expressing *cry2Ae* gene that confers resistance to certain lepidopteran pests and the *pat* gene that confers tolerance to herbicide products containing glufosinate ammonium;
- MON 89034, expressing the *cry1A.105* and *cry2Ab2* genes for resistance to certain lepidopteran pests;
- 5307, expressing the *eCry3.1Ab* gene for resistance against certain coleopteran pests and the *pmi* gene that is used as selectable marker; and
- GA21, expressing the double-mutated *mepsps* gene that confers tolerance to herbicide products containing glyphosate.

The application was validated by EFSA on 6 July 2018. A formal three-month consultation period of the Member States was started, lasting until 15 October 2018, 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). Two experts answered positively to this request. See Annex I for an overview of the comments forwarded to EFSA on 12 October 2018.

The opinion of the EFSA Scientific Panel on GMOs, including the responses from the EFSA GMO Panel to comments submitted by the Member States during the three-month consultation period, was published on 5 June 2023 (EFSA Journal 2023;21(6):8011[2]).

[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.2023.8011

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In delivering the present advice, the BAC considered in particular the following information:
- The feedback of the experts on application EFSA-GMO-DE-2018-149;
- The opinion of EFSA on application EFSA-GMO-DE-2018-149;
- The advices already adopted by the BAC on the single events and the lower-order stacks[3]. The conclusions of the BAC for the most recent applications for the single events and the lower-order stacks were as follows:

Event	Application number	BAC advice	Conclusions
Bt11	RX/016	BAC/2021/0063	No major risks for human and animal health or concerning the environment were identified.
MIR162	RX/025	BAC/2022/1181	Unlikely to pose any risk to human and animal health. No risk identified for the European environment.
MIR604	UK-2010-83	BAC/2016/0789	No conclusive advice as no conclusive advice on human health (i.e. allergenicity potential of PMI protein remains an issue).
	RX/013	BAC/2019/1084	Unlikely to pose any risk to human and animal health. No risk identified for the European environment.
MON 89034	NL-2007-37	BAC/2009/880	Unlikely to pose any risk to human and animal health. No risk identified for the European environment.
5307	DE-2011-95	BAC/2018/0327	Unlikely to pose any risk to human and animal health. No risk identified for the European environment.
GA21	UK-2008-60	BAC/2012/0216	Unlikely to pose any risk to human and animal health. No risk identified for the European environment.
Bt11 x GA21	UK-2007-49	BAC/2009/01493	Unlikely to pose any risk to human and animal health. No risk identified for the European environment.
Bt11 x MIR604	UK-2007-50	BAC/2010/0956	No conclusive advice on human health (as remaining question for MIR604, see UK-2010-83)
MIR604 x GA21	UK-2007-48	BAC/2010/0952	No conclusive advice on human health (as remaining question for MIR604, see UK-2010-83)
Bt11 x MIR604 x GA21	UK-2008-56	BAC/2010/958	No conclusive advice on human health (as remaining question for MIR604, see UK-2010-83)
Bt11 x MIR162 x MIR604 x GA21	DE-2009-66	BAC/2016/0122	Unlikely to pose any risk to human and animal health (i.e. issues with allergenicity potential of PMI protein were resolved). No risk identified for the European environment.

[3] This list is not exhaustive at the level of lower-order stacks already assessed, but covers all the applications covering lower-order stacks and for which the BAC issued an advice. For an exhaustive list of all the lower-order stacks already assessed, we refer to the EFSA opinion.

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

Taking into account the previous assessment of the single events and the new compositional data provided by the applicant for the six-stacked event, the Biosafety Advisory Council agrees with the GMO panel of EFSA that the compositional data of GM maize six-stacked event, 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 newly produced Cry1A.105, Cry1Ab, Cry2Ab2, Cry2Ae, eCry3.1Ab, Vip3Aa20, PMI, mEPSPS and PAT proteins in the context of previous applications, and no safety concerns with respect to toxicity were identified, beyond the intended toxicity of the insecticidal proteins to their target pests. Taking into account the updated information considered in the current application, the Council is of the opinion that its previous conclusions remain valid.
Based on the known biological functions, the Biosafety Advisory Council is also of the opinion that the combined presence of the newly expressed proteins in the six-stacked maize event does not raise toxicological concerns.

2.3. Assessment of allergenicity

The Biosafety Advisory Council has evaluated the safety of the newly produced Cry1A.105, Cry1Ab, Cry2Ab2, Cry2Ae, eCry3.1Ab, Vip3Aa20, PMI, mEPSPS and PAT proteins in the context of previous applications, and no concerns with respect to allergenicity were identified. Since no new information on allergenicity of these proteins has become available, the Council is of the opinion that its previous conclusions remain valid.
The Biosafety Advisory Council is also of the opinion that the combined presence of the newly expressed proteins in the six-stacked maize event does not raise concerns regarding the 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 the food and feed derived from the six-stacked maize event 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[4]; COGEM, 2011[5]; Pascher, 2016[6]). 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[7]). However, volunteer maize has been shown to grow

[4] 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

[5] 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/

[6] 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

[7] 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/

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 Bt11 x MIR162 x MIR604 x MON 89034 x 5307 x GA21 (i.e. during transport and/or processing) into the European environment[8] 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 Bt11 x MIR162 x MIR604 x MON 89034 x 5307 x GA21 provided by the applicant, the scientific assessment of the dossier done by the Belgian experts, the opinion of EFSA, the answers of the EFSA GMO panel to the questions raised by the Belgian experts, and the advices already adopted by the BAC on the single events and lower-order stacked events, the Biosafety Advisory Council:

1) Agrees with the GMO panel of EFSA that the potential environmental release of the six-stacked maize event is unlikely to pose any threat to the European environment;
2) Agrees with the GMO panel of EFSA that there is no reason to expect interactions between the newly expressed proteins that could impact on the food or feed safety;
3) Agrees with the GMO panel of EFSA that in the context of its proposed uses, six-stacked maize event is unlikely to pose any risk to 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

[8] 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 EFSA/GMO/DE/2018/149 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

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Experts: Jacques Dommes (ULg), Jan Van Doorsselaere (Vives)
SBB: Adinda De Schrijver

Application: **EFSA/GMO/DE/2018/149**

Applicant: **Syngenta**

GMO: **maize Bt11 X MIR162 X MIR604 X MON89034 X 5307 X GA21**

Acknowledgement of receipt by EFSA: **6 July 2018**

Scope of the application:
☒ Food containing or consisting of GM plants
☒ Food produced from GM plants or containing ingredients produced from GM plants
☒ Feed containing or consisting of GM plants
☒ Feed produced from GM plants
☒ Products other than food and feed containing or consisting of GM plants with the exception of cultivation
☒ 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)

As this application concerns a stacked event, and all the single events and lower order stacks have previously received a positive advice from the Council, the Biosafety Advisory Council decided to evaluate only the specific risk assessment aspects linked to the stacked as mentioned in the Commission Implementing Regulation (EU) No 503/2013, i.e. stability of the traits, expression of the new proteins, and interactions between the newly expressed traits.

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.

No comments were received from the consulted experts on AP149. However, the SBB made some comments on the general approach of the risk assessment. Comments placed on the EFSAnet are mentioned in the Annex.

ANNEX – COMMENTING FORM

GENERAL COMMENTS

We do not have any comments and we consider all the necessary information is present to conduct a robust risk assessment.

SBB comment:

We noticed that during the consultation period with the Member States, EFSA has asked for additional information on (1) the agronomic and phenotypic studies that have been carried out in the context of AP149 and (2) the dietary exposure of humans to the newly expressed proteins. In our view, these are 'nice to know information' requests that are not needed to conclude on the risk assessment:

Concerning the **agronomic and phenotypic studies**, we are of the opinion that additional information on such studies should only be requested if there is a clear hypothesis for the need of such studies. In case of this particular stack, expressing herbicide-tolerant and insect-resistant traits, we do not see how agronomic and phenotypic field studies can inform the food/feed risk assessment and therefore we ask the EFSA GMO Panel to explain for which hypothesi(e)s (and corresponding pathway(s) to harm) they consider the additional requested information relevant.

For a better understanding of the question above, we want to clarify, that we do not consider agronomic and phenotypic field studies as part of the basic package that should be handed in for the assessment of GM stacked events (as is the case for single GM events). For stacks where no interaction is anticipated between the GM events, such studies will not reveal unintended effects that are a result of the genetic modification (which can be done at the level of the single event), but will rather reveal unintended effects as a result of conventional breeding. Thus, at this level we agree with EFSA that the evaluation of a GM stack event can focus on (a) stability of the insert, (b) expression of the proteins and (c) potential synergistic or antagonistic effects resulting from the combination of the events (EFSA Journal 2011; 9(5):2150, p.8).

Concerning **dietary exposure to humans**, we wonder what the trigger is to ask for this evaluation and ask the EFSA GMO Panel to clarify their scientific reasoning? As no hazard has been identified for any of the traits of this 6-event stack (for this we refer to the previous opinions of EFSA on the single events), one can ask what the added value is of exposure data to characterize the risk? Whether the exposure is low, medium or high, the risk (hazard * likelihood of exposure) will remain low due to a low (no) hazard.

Further, we note that such a detailed exposure assessment has not been requested by EFSA in the risk assessment of the single events (as no hazards were identified). We therefore also ask the EFSA GMO Panel to explain why such an assessment is now considered needed for the GM stacked event containing exactly the same traits as the single events and in which the expression levels for all the traits, except the PMI protein, have been shown to be similar between single events and the stack product?

Reply EFSA

The EFSA Panel thanks Belgium for sharing its view.
The requested information (1) was not considered a nice to know but needed to allow the Panel to conclude on the quality and representativeness of the field trials that were used to build one of the main pillars of the RA that is the comparative analysis. The position of the GMO Panel is reported in the guidance on the agronomic and phenotypic characterisation of the GM plants (EFSA GMO Panel, 2015a).

Concerning human dietary exposure (2), in the dossier submitted to EFSA for the risk assessment of Bt11 x MIR162 x MIR604 x MON 89034 x 5307 x GA21 maize, a study report (SSB-244-17) on human dietary exposure to newly expressed proteins (Cry1Ab, Vip3Aa20, mCry3A, Cry1A.105, Cry2Ab2, eCry3.1Ab, mEPSPS, PAT and PMI proteins) was originally included. The submission of data on exposure assessment/ anticipated intake is a requirement established by Regulation (EU) No 503/2013 (section 2, EXPOSURE ASSESSMENT – ANTICIPATED INTAKE/EXTENT of USE, page 40).
During the assessment, the GMO Panel noticed that in the above-mentioned study report, the GM maize was not treated with the intended herbicides (glufosinate ammonium and glyphosate herbicides). As indicated in section 2 of the Regulation (EU) No 503/2013 "the expected range of concentrations of newly produced proteins or existing plant proteins deliberately modified in the genetically modified food(s) and feed(s) to be placed on the market shall be provided". The GMO Panel considered that the GM stack maize that will enter the market will be the one treated with the intended herbicides, as the GM stack contains different traits that makes it tolerance to these herbicides. Therefore, the GMO Panel asked the applicant to submit information on the expression levels of the newly expressed proteins in different parts of the GM plant treated with the intended herbicides (for humans, in kernels and pollen), and to use these expression levels to estimate human dietary exposure.
The expression levels in the treated GM stack maize informed, additionally, on possible effects of the combined used of herbicides by comparing these expression levels with those reported for the single events. The risk assessment of the single events part of the Bt11 x MIR162 x MIR604 x MON 89034 x 5307 x GA21 maize were not done under Regulation (EU) No 503/2013 and, therefore, some of the information required now as part of the risk assessment of the GM stack might have not been asked for the single events.