Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on application EFSA-GMO-NL-2018-150 (genetically modified maize DP4114 x MON810 x MIR604 x NK603 and subcombinations) from Pioneer under Regulation (EC) No. 1829/2003

02 June 2022
Ref. SC/1510/BAC/2022_0697

Context

Application EFSA-GMO-NL-2018-150 was submitted by Pioneer for the marketing of genetically modified (GM) maize DP4114 x MON810 x MIR604 x NK603 (Unique Identifier DPØØ4114-3, MON-ØØ81Ø-6, SYN-IR6Ø4-5, MON-ØØ6Ø3-6) and its subcombinations, 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 four-event stack maize DP4114 x MON810 x MIR604 x NK603 was obtained by conventional crossing (no new genetic modification involved) of the corresponding single events:
- DP4114, expressing the Cry1F, Cry34Ab1 and Cry35Ab1 proteins for resistance against lepidopteran and coleopteran pests, and the PAT protein for tolerance to glufosinate-ammonium herbicides;
- MON810, expressing the Cry1Ab protein to confer resistance to lepidopteran pests;
- MIR604, expressing the mCry3A protein to confer resistance to coleopteran pests and PMI as selectable marker;
- NK603, expressing the CP4 EPSPS protein and its variant CP4 EPSPS L214P, for tolerance to glyphosate;

The application was validated by EFSA on 10 August 2018. A formal three-month consultation period of the Member States was started, lasting until 12 November 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). 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 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. 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 and the comments forwarded to EFSA on 3 October 2018.

The opinion of the EFSA Scientific Panel on GMOs was published on 7 March 2022 (EFSA Journal 2022;20(3):7134[2]), together with the responses from the EFSA GMO Panel to comments submitted by the Member States during the three-month consultation period.

[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.2022.7134

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In delivering the present advice the BAC considered in particular the following information:
- The comments formulated by the experts on application EFSA-GMO-NL-2018-150;
- The opinion of EFSA;
- The advices already adopted by the BAC on the single events and one previously assessed subcombination. The conclusions of the BAC for the most recent applications for the single events and subcombination were as follows:

Event	Application number	BAC advice	Conclusions
DP4114	EFSA-GMO-NL-2014-123	BAC_2018_0463 (03/07/2018)	Unlikely to pose any risk to human and animal health and the environment
MON810	EFSA-GMO-RX-MON810	BAC_2009_1510 (17/11/2009)	No major risks for human and animal health or concerning the environment were identified.
MIR604	EFSA-GMO-RX-013	BAC_2019_1084 (10/12/2019)	Unlikely to pose any risk to human and animal health and the environment
NK603	EFSA-GMO-RX-NK603	BAC_2009_1367 (02/10/2009)	No major risks for human and animal health or concerning the environment were identified.
MON810 x NK603	EFSA-GMO-RX-007	BAC_2018_0215 (17/04/2018)	Unlikely to pose any risk to human and animal health. No risk identified for the European environment

All GM maize events mentioned in the table above are authorised in the EU for food and feed uses[3].

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 data on compositional analysis provided by the applicant for the four-stacked event, the Biosafety Advisory Council agrees with the GMO panel of EFSA that the compositional data of GM maize DP4114 x MON810 x MIR604 x NK603, 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 expressed Cry1F, Cry34Ab1, Cry35Ab1, Cry1Ab, mCry3A, PMI, CP4 EPSPS, CP4 EPSPS L214P and PAT proteins in the context of previous applications, and no safety concerns were identified. Taking into account the updated information considered in the current application, the Council is of the opinion that its previous conclusions remain valid.
The Biosafety Advisory Council is also of the opinion that the combined expression of the newly expressed proteins in the stacked event does not raise toxicological concerns. The five insecticidal proteins are delta-toxins for which in the gastro-intestinal tract of mammals, including humans, no specific affinity receptors are present. The three enzymatic proteins catalyse distinct biochemical reactions, acting on unrelated substrates and are not expected to interact.

2.3. Assessment of allergenicity

The Biosafety Advisory Council has evaluated the safety of the newly expressed Cry1F, Cry34Ab1, Cry35Ab1, Cry1Ab, mCry3A, PMI, CP4 EPSPS, CP4 EPSPS L214P and PAT proteins in the context of previous applications, and no concerns were identified. Since no new information on allergenicity of

[3] See EU register of GM food and feed: http://ec.europa.eu/food/dyna/gm_register/index_en.cfm

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 expression of the newly expressed proteins in the stacked event does not raise concerns regarding the allergenicity. Based on the current knowledge, and as there is no evidence of allergenicity of the newly expressed proteins, there are no concerns of allergenicity as a consequence of their joint presence.

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 DP4114 x MON810 x MIR604 x NK603-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[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 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 DP4114 x MON810 x MIR604 x NK603 (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.

[4] Palaudelmàs M, Peñas G, Melé E, et al. Effect of volunteers on maize gene flow. Transgenic Res. 2009;18(4):583-594. doi:10.1007/s11248-009-9250-7
[5] COGEM 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". 2011. 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. Spread of volunteer and feral maize plants in Central Europe: recent data from Austria. Environ Sci Eur. 2016;28(1):30. doi:10.1186/s12302-016-0098-1
[7] COGEM research report "Are Teosinte and Feral Maize present in the Netherlands?". 2018. https://cogem.net/en/publication/are-teosinte-and-feral-maize-present-in-the-netherlands/
[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.

Conclusion

Based on the whole set of data on maize DP4114 x MON810 x MIR604 x NK603 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 four single events and one subcombination, the Biosafety Advisory Council:

1) Agrees with the GMO panel of EFSA that the potential environmental release of maize DP4114 x MON810 x MIR604 x NK603 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, maize DP4114 x MON810 x MIR604 x NK603 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

Annex : Outcome of the assessment of application EFSA/GMO/NL/2018/150 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: René Custers
Experts: Jacques Dommes (ULg), Frank Van Breusegem (UGent), Jan Van Doorsselaere (Vives)
SBB: Fanny Coppens

Application: **EFSA/GMO/NL/2018/150**
Applicant: **Pioneer**
GMO: **maize DP4114 x MON810 x MIR604 x NK603**
Acknowledgement of receipt by EFSA: **10 August 2018**

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

Remark: 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 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.

Comments sent to EFSA are indicated in grey. 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 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: 2 experts

1.2. MOLECULAR CHARACTERISATION

1.2.1. Information relating to the genetic modification

Have evaluated this section and consider the information adequate: 3 experts

1.2.2. Information relating to the genetically modified plant

Have evaluated this section and consider the information adequate: 3 experts

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: 3 experts

1.2.4. Conclusions of the molecular characterisation

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

Comment 1

The wordings used in the conclusions on p43-44 are sometimes rather awkward or incorrect. In addition, a more consistent description of the analyses (related to the insert position in the genome) for each single event is recommended.
E.g. MON810: A) What is the evidence/argumentation that the mentioned HECT Ubiquitin ligase gene is “not biologically relevant” ? The authors mean that the interruption of this gene has no functional consequences that are relevant towards risk/hazard. This is fine, but the statement that this gene is “biologically irrelevant” is not correct. B) The argumentation: “Moreover, there is no known physiological role..” cannot be used as an argument to classify the protein as irrelevant. C) What is meant with “no biologically significant protein” produced ?

DP4114: In contrast to MON810 were it is stated that the insertion is located at chromosome5, for DP4114 it is described as “very high likelihood” to be located on chr5.

For MIR604: no chromosome location is described.

P45. NK603 (ORFs): Why is it mentioned “in the unlikely occurrence that these peptides are expressed” ?

Coordinator comment sent to EFSA:

We wish to make the following minor remarks on the conclusions of the molecular characterisation, even though they do not change our views on the safety of this GM maize:

- From the information available in the dossier it cannot be concluded that the HECT Ubiquitin ligase gene is 'not biologically relevant'.
- For MIR604 the chromosomal location of the insert is not described.
- We note a difference in the certainty presented about the chromosomal location of the inserts. For MON810 it is stated that the insertion is located at chromosome5, where for D4114 the insert location is described as 'very high likelihood' to be located on chromosome5. Based on the evidence presented we see no reason for differing certainties.

Feedback from the EFSA GMO Panel: The maize HECT Ubiquitin ligase gene which is present at the 3' of the MON810 insert has been previously risk assessed in the frame of the renewal application EFSA-GMO-RXMON810 and no safety implications regarding the interruption of this gene were identified by the GMO panel experts (EFSA, 2009). In the frame of stack applications, the Implementing Regulation (EU) N.503/2013 requires the applicant to verify that the integrity of the event has been maintained between the original transformation event (single) and the stacked transformation event using material representative for commercial production. The applicant provided the sequencing analysis for all the four inserts and flanking regions in the stack material demonstrating that no nucleotide change has occurred in the stacked events compared to the events in the respective singles. No requirements are laid down in the Implementing Regulation as to provide the exact location of the events in the plant genome. Nonetheless, the flanking regions (5'- and 3'-flank) of each insert in the stack are identical to the flanking regions in the events from the respective singles. This demonstrates that the genomic context where the inserts have been integrated is maintained between the stack and the singles.