Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on application EFSA-GMO-NL-2019-163 (maize DP23211) from Pioneer under Regulation (EC) No. 1829/2003

12 March 2024
Ref. SC/1510/BAC/2024_0349

Context

Application EFSA-GMO-NL-2019-163 was submitted by Pioneer Hi-Bred International for the authorisation for the marketing of genetically modified (GM) maize DP23211 (Unique Identifier DP-Ø23211-2) for food and feed uses, import and processing (excluding cultivation) within the European Union, within the framework of Regulation (EC) No. 1829/2003[1].

DP23211 contains a single insert consisting of one copy of the *mo-pat*, DvSSJ1 dsRNA, *ipd072Aa* and *pmi* cassettes, expressing the PAT protein (tolerance to glufosinate), the DvSSJ1 double-stranded RNA (dsRNA) and the IPD072Aa protein (both for control of corn rootworm pests), and the phosphamannose isomerase (PMI) protein (used as a selectable marker), respectively.

The application was validated by EFSA on 17 April 2020 and a formal three-month consultation period of the Member States was started, lasting until 17 July 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 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 6 July 2020.

The opinion of the EFSA Scientific Panel on GMOs was published on 18 January 2024 (EFSA Journal 2024;22(1):e8433[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 25 January 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 EFSA-GMO-NL-2019-163 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.2024.8483

Biosafety Advisory Council - Secretariat • Service Biosafety and Biotechnology (SBB)
Sciensano • Rue Juliette Wytsmanstraat 14 • B-1050 Brussels • Belgium
T + 32 2 642 52 93 • bac@sciensano.be • www.bio-council.be

Scientific evaluation

1. Molecular characterisation

After request for more information on the potential expression of newly identified ORFs within the DP23211 insert and junction regions between the insert and genomic DNA that showed sequence similarities to allergens above the set threshold levels by EFSA, the Biosafety Advisory Council is of the opinion that the molecular characterisation data do 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 DP23211, 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 PAT and PMI protein in the context of previous applications, and no concerns with respect to toxicity were identified. Since no new information on the toxicity of these proteins has become available, the Council is of the opinion that its previous conclusions remain valid.

The Biosafety Advisory Council evaluated the safety of the newly produced IPD072Aa protein and no safety concerns with respect to toxicity were identified.

The Biosafety Advisory Council is also of the opinion that the combined presence of the newly expressed proteins in DP23211 does not raise toxicological concerns..

2.3. Assessment of allergenicity

The Biosafety Advisory Council has evaluated the safety of the newly expressed PAT and PMI protein 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 evaluated the safety of the newly produced IPD072Aa protein 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 DP23211 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 DP23211-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 DP23211 (i.e. during transport and/or processing) into the European environment[7] will lead to environmental harm.

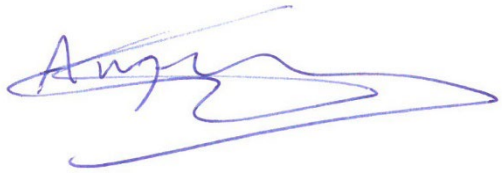
4. Monitoring

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 DP23211 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 DP23211 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 DP23211 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

[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.

Annex : Outcome of the assessment of application EFSA/GMO/NL/2019/163 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: **EFSA/GMO/NL/2019/163**
Applicant: **Pioneer Hi-Bred International, Inc.**
GMO: **maize DP023211**
Validation of dossier by EFSA: **17 April 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.

Comments sent to EFSA are highlighted in grey, with the answers from the GMO Panel from EFSA provided underneath.

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: 2 experts

1.2. MOLECULAR CHARACTERISATION

1.2.1. Information relating to the genetic modification

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

1.2.2. Information relating to the genetically modified plant

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

Comment:

1. On page 36 of the main dossier, the applicant summarizes the search for similarities to allergens within the DP23211 insert. Following the guidelines of EFSA, sequences exceeding the threshold were found and further assessed, which included the assessment of the likelihood of expression. However, how this was performed and using which bioinformatic criteria, could not be found in the dossier or in Annex 8. Is this only based on the search for putative translation start codons? The applicant should be more detailed and convincing here.

2. The expert acknowledges the in-depth analysis, following a clear rationale and complying with EFSA guidelines, of the RNAi off target assessment. Surprisingly, the results are summarized in page 39, in the section "1.2.2.3 Information on the expression of the insert" and in Annex 12, though this is clearly not about the expression of the insert. The current guidelines and implementing regulation seems to be unclear about where to include such piece of information within the dossier. This remark is more for EFSA and the COM than for the applicant.

3. For the assessment of the potential unintended expression of new ORFs identified, the information is split between pages 36 and 49, the text is very brief and refers to annex 9. However Annex 9 only contains the raw search reports and it is difficult for experts looking at the dossier to figure out how the analysis was actually performed (number of new ORFs, origin and years of databases, etc.), without opening the dozens of files contained in the folder Annex 9. This is not acceptable.

Feedback from the EFSA GMO Panel: The GMO Panel takes note and thanks Belgium for the comment. EFSA requested bioinformatic updated analyses which were provided by the applicant with additional information received on 15/9/2023. These analyses included the analysis to identify whether open reading frames (ORFs) present within maize DP23211 insert and spanning the junctions between the insert and the flanking genomic DNA, potentially encode peptides that match with regions of known allergenic proteins. The applicant identified some hypothetical ORFs sharing significant similarities to allergens according to EFSA guidances. These ORFs are described in Section 3.3.2 of the Scientific Opinion. The GMO Panel assessed the likelihood of expression of these hypothetical ORFs taking into consideration the genomic contexts characterizing these ORFs. Giving the absence of any genetic element that could promote their expression, like promoters, position of start codons, direction of putative expression, the GMO Panel concluded that the expression of these ORFs is unlikely.

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: 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: 4 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: 3 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 consensus document of 2002 is followed. Constituents analysed are summarized in a list for forage and for grain. My comments apply to grain and not to forage.

Although the OECD document is followed the information on carbohydrates and fibre does not meet the actual insight in the composition of food for human nutrition. The acid and neutral detergent approach is not used at all in human nutrition, but for animal nutrition.

On the other hand the information is adequate for the amino acid, fatty acid, mineral, vitamin composition as well for secondary metabolites and anti-nutrients.

With respect to tocopherols additional information is given. The OECD document mentions only vitamin E or α-tocopherol. The other tocopherols isomers have been analyzed. I consider this as very important taking into account the high unsaturation of maize oil and the stabilization by natural antioxidants.

1.3.4. Comparative analysis of composition

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

Comment 1:

Analytes are categorized according to the general applied system for statistical analysis. The biological significance of analytes classified as category III and IV is further discussed. Box plot representations are used to visualize the obtained results. If any significant difference is observed, it is demonstrated that the variation is within the natural range of biological variation of maize. This approach is used among others for amino acids, mineral and phytic acid. Phytic acid is an important anti-nutrient. The applicant concludes that the composition of maize 163 is comparable to conventional maize. I agree with this conclusion.

Comment 2:

For phytic acid in grain, I do not understand the following sentence: "….*some samples were above the tolerance interval. However, all values (for phytic acid) fell within the literature range of not detectable to 1.940% DW*". But in table 22, levels of phytic acid as % DW in grain range from 0.694 to 1.38 % DW, so much lower than the so called not detectable to 1.940% DW?? What did I misunderstand?

Note coordinator & SBB: The sentence should be interpreted as follows: values found for phytic acid in literature range from 0 up to 1.940 %DW.

I can agree with the conclusion that the results of the compositional analysis demonstrated that nutrient composition of forage and grain derived from DP23211 maize was comparable to that of conventional maize represented by the non-GM near-isoline control as well as to non-GM conventional maize.

1.3.5. Comparative analysis of agronomic and phenotypic characteristics

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

Comment:
The applicant lists the tests performed (table 23 on page 77 of the main dossier) and puts in Annex 17 the files with the methods and raw data. But we don't see (at least I could not see) the conclusions of the applicant in a summary report or in the main text. This should have been done.

Note coordinator & SBB: In our view, the conclusions are written down in the form of a table and a short text at the end of p. 77 of the main dossier, concluding that "*The results for the agronomic characteristics were comparable between DP23211 maize and those of conventional maize represented by non-GM near-isoline control maize and non-GM commercial maize*".

1.3.6. Effects of processing

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

Comment:
It is concluded that it is unlikely that the processes will be affected by the introduction of the traits in maize 163. I agree with this conclusion.

1.3.7. Conclusion

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

Comment 1:
I agree with the overall conclusion by the applicant.

Comment 2:
Please consider my remark above.

1.4. TOXICOLOGY

1.4.1. Testing of newly expressed proteins

Comment 1:
Pseudomonas chlororaphis, as a common inhabitant of the root environment, may stimulate or promote plant growth by stimulating microbial communities and protect plants by producing compounds that inhibit fungal growth, insects and nematodes. But does *P. chlororaphis* also directly promote plant growth as is suggested in this paragraph by the way it is formulated? This is not clear to me.

Note coordinator & SBB: We do not see this as a necessary question for information to come to a risk/safety conclusion.

Under the section 'acute toxicity in mice for IPD072Aa protein' (p.83 of main dossier), the dose was adjusted for IPD072Aa protein concentration of 820 microgram/mg? I do not understand this formulation; 820 microgram / mg of what?
The control groups were dosed by oral gavage with either deionized water or 2000mg/kg BSA?? I guess it is ment 2000mg/kg of body weight of BSA, or 2000mg BSA/kg BW???

Note coordinator & SBB: To our understanding, this is explained in Annex 22 (p.13): *Individual dose volumes were calculated based on the individual fasted body weights determined on the day of dosing*. However, this could have been more clearly formulated in the main text.

Feedback from the EFSA GMO Panel: The GMO Panel thanks Belgium for the comment. The dose was adjusted for IPD072Aa protein concentration of 820 microgram of protein per mg of lyophilised powder (please refer to the certificate of analysis on page 24 of the Annex 22 (acute study report).

The modification of PAT and toxicological evaluation of PAT and PMI are well explained.

Comment 2:
The bioinformatic part was assessed before.

Comment 3:
28-day repeated dose for IPD072Aa: For both the high group female and high group male, an inflammatory response was observed. As no such effect is observed in the 90-day rat feeding study, this seems to be of no concern.

1.4.2. Testing of new constituents other than proteins

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

Comment:
I understand that the safety assessment of DvSSJ-1 ds RNA begins with an understanding of its functional activity and molecular target, a portion of the smooth septate junction protein-1 gene from WCR. Since smooth septate junctions are unique to invertebrates (with role in intestine and renal system) this is extremely important for the safety assessment in food and feed systems involving vertebrates (man and domestic animals).
However, the species specificity for invertebrates is important for ecosystem safety assessment. Therefore, the spectrum of activity of DvSSJ-1 ds RNA was assessed in bioassays with 10 species from 4 families of coleopteran and 4 species of 4 families of lepidoptera. This involves only 2 orders.
Of the about 23 orders of the subphylum of insect only, and in terms of number of families or species, this is a minimal number. What about other phyla or subphyla of invertebrates?
What is the meaning of the sentence (citation of Boeckman 2019) that “concentrations up to 1 ppm represented dietary concentrations at least 10-fold higher than those expected for each species under normal conditions on the ecosystem” ?
What are these doses expected in the agroecosystem? What are “normal” conditions? For each species? Of what???

Conclusion: is it sure that the molecular target of DvSSJ-1 dsRNA is arthropod-specific? And is it specific to Diabrotica species? If only 2 orders of insect are tested, and not any member of the subphylum of crustacean, how can it be stated then that it is arthropod-specific (Phylum arthropoda, consisting of 2 subphyla: crustacean and insect.
What is known about the presence of SSJ-proteins in other phyla, classes and orders of invertebrata?

Note coordinator & SBB: The available information on the DvSSJ-1 dsRNA provided points into the direction that this dsRNA is arthropod specific and particularly acts against Diabrotica. The knowledge that this protein acts against WCR is most likely used as a starting point to assess closely related insects (in the order of Coleoptera) and subsequently known other pests of maize (in the order of Lepidoptera). A weight of evidence approach is taken here, rather than stating one is 100% sure.
As the application is only meant for import and processing, we do not see the need to ask for more information on the specificity of the dsRNA towards arthropods.

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: 2 experts

1.4.5. Conclusion of the toxicological assessment

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

1.5. ALLERGENICITY

1.5.1. Assessment of allergenicity of the newly expressed protein

Comment:
The bioinformatic part was assessed before.

1.5.2. Assessment of allergenicity of the whole genetically modified plant

No comments received

1.5.3. Conclusion of the allergenicity assessment

No comments received

1.6. NUTRITIONAL ASSESSMENT

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

2. EXPOSURE ASSESSMENT — ANTICIPATED INTAKE OR EXTENT OF USE

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

3. RISK CHARACTERISATION

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

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

5. ENVIRONMENTAL RISK ASSESSMENT (ERA)

5.1. INTRODUCTION

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

5.2. GENERAL APPROACH OF THE ERA

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

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: 1 expert

5.3.2. Plant to micro-organisms gene transfer

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

Comment:

Nowhere in the dossier, the presence and possible relevance of the single loxP site in the insert was commented by the applicant. Considering the function of the lox sequences in site-specific recombination in bacteria, the applicant should have discussed the possible impact of this sequence on the genetic stability and HGT of the insert to bacteria.

Note coordinator & SBB: The applicant makes reference to a previous assessment of EFSA when it come to the Cre-lox system: *The presence of site-specific recombination sites has previously been assessed by EFSA (EFSA, 2012e) and the Panel concluded that enhanced horizontal transfer of the nptII gene due to Cre-lox-mediated recombination is unlikely* (p.129 of the main dossier).

5.3.3. Interactions of the GM plant with target organisms

No comments received

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

Comment 1:
See comment on 1.4.2

Comment 2:
NB: an important part of the assessment of the possible effects on NTOs of the dsRNAs from event DP23211 is developed in “section 1.4.2. Testing of new constituents other than proteins” from the toxicology part, which the applicant should have referred to here.

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

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

5.3.6. Effects on biogeochemical processes

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

5.3.7. Effects on human and animal health

Have evaluated this section and consider the information adequate: Patrick du Jardin

5.3.8. Overall risk evaluation and conclusions

Have evaluated this section and consider the information adequate: Eddy Decuypere

6. POST-MARKET ENVIRONMENTAL MONITORING PLAN (PMEM)

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

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