Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on application GMFF-2021-1530 (maize DAS1131) from Corteva under Regulation (EC) No. 1829/2003

12 May 2025
Ref. SC/1510/BAC/2025_0632

Context

Application GMFF-2021-1530 was submitted by Corteva AgriScience for the authorisation for the marketing of genetically modified (GM) maize DAS1131 (Unique Identifier DAS-Ø1131-3) for food and feed uses, import and processing (excluding cultivation) within the European Union, within the framework of Regulation (EC) No. 1829/2003[1].

DAS1131 maize was genetically modified to produce the Cry1Da2 protein from *Bacillus thuringiensis* for control of certain lepidopteran insect pests and the DGT-28 EPSPS protein from *Streptomyces sviceus* for tolerance to glyphosate herbicide.

The application was validated by EFSA on 9 January 2023 and a formal three-month consultation period of the Member States was started, lasting until 10 April 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). Five 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 28 March 2023.

The opinion of the EFSA Scientific Panel on GMOs was published on 19 March 2025 (EFSA Journal 2025;23:e9282[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 8 March 2025, 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-2021-1530 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.2025.9282

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Scientific evaluation

1. Molecular characterisation

Maize DAS1131 contains a single insert consisting of one copy of the *cry*1Da2 and *dgt-28 epsps* expression cassettes. 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 DAS1131, in comparison with its conventional counterpart, do not raise safety concerns.

2.2. Assessment of toxicity

Two proteins, Cry1Da2 and DGT-28 EPSPS, are newly expressed in maize DAS1131. These proteins have not been previously assessed by the Biosafety Advisory Council. The Cry1Da2 protein is derived from *B. thuringiensis*, however, Cry1Da2, as expressed in this event, is not a naturally occurring gene, but rather a designed chimeric construct. The DGT-28 EPSPS protein belongs to a newly discovered Class IV EPSPS. No safety concerns with respect to toxicity were identified.

The Biosafety Advisory Council is of the opinion that the combined presence of these newly produced proteins in DAS1131 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 DAS1131, in comparison with its conventional counterpart, does not raise safety concerns.

2.3. Assessment of allergenicity

The Biosafety Advisory Council evaluated the safety of the newly produced Cry1Da2 and DGT-28 EPSPSP proteins and no safety concerns with respect to allergenicity were identified.

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 DAS1131-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 DAS1131 (i.e. during transport and/or processing) into the European environment[7] will lead to environmental harm.

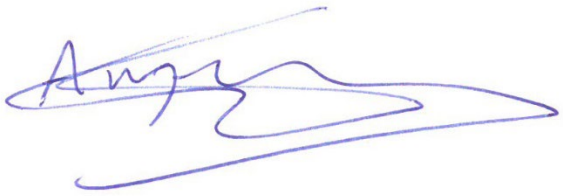
4. Monitoring

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 DAS1131 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 DAS1131 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 DAS1131 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-2021-1530 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)

Coordinator: Prof. Lieve Gheysen
Experts: Henri Batoko (UCL), Dimitri Gilis (ULB), André Huyghebaert (UGent), Frank Van Breusegem (UGent), Erik Van Miert (Sciensano)
SBB: Adinda De Schrijver

Application: **GMFF-2021-1530 (AP175)**
Applicant: **Corteva Agriscience**
GMO: **maize DAS1131**
Validation of dossier by EFSA: **9 January 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
☐ 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. The comments indicated in grey in Annex I were sent to EFSA. 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 safety issues covered under other EU regulation, and statements on GMOs (e.g. socio-economic considerations) or statements without supporting reasoning or evidence.

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

PART II - SCIENTIFIC INFORMATION

1. HAZARD IDENTIFICATION AND CHARACTERISATION

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

1.2. MOLECULAR CHARACTERISATION

1.2.1. Information relating to the genetic modification

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

Comment:
In the summary document, it is stated that the construct contains 3 gene cassettes (p. 13). However, to my opinion and according to the remaining information (e.g. on p.1 in Appendix B6) only two cassettes are present (expressing Cry1Da2 and dgt-28 EPSPS). Unless the landing platforms and ZFN sites are considered as cassettes.

Minor comment: p.17 in reference Barry, the year of publication is missing.

Note SBB: Proposals for textual changes are not considered at this stage of the evaluation process.

1.2.2. Information relating to the genetically modified plant

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

Comment:
Bioinformatics analysis of the allergenicity and toxicity are partly done in section 1.2.2, but also in sections 1.4 and 1.5. I will give all my comments in these latter sections.

1.2.3. Conclusions of the molecular characterisation

Have evaluated this section and consider the information adequate: 3 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: 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: 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 was followed: no basic remarks

Results include:
- Forage proximates
- Grain: proximates dietary fiber not differentiated according to actual insights
- Fatty acids
- Amino acids
- Minerals
- Vitamins: the whole range of B vitamins is included; the four members of tocopherols, vit E equivalents are analysed: alpha, beta, gamma and delta. The equivalent tocotrienols, however, are not considered. They have a more potent antioxidant activity than tocopherols. Data on those compounds would strengthen the conclusions about equivalence and stabilization against oxidation.
- Secondary compounds: particular attention is given to compounds with a potential negative effect.
- Data on the, in maize, important carotenoids would be welcome.
- In the OECD document important groups of toxicants are not mentioned: mycotoxins and heavy metals. Both groups receive a lot of attention in the debate of food safety. Both issues are briefly discussed in 1.3.5 agronomic and phenotypic characteristics.

Note SBB & coordinator: The components analysed by applicants are based on the crop documents of the OECD. Tocotrienols and carotenoids are not included in the list of components to be analysed for maize according to the OECD (https://www.oecd.org/env/ehs/biotrack/46815196.pdf). The OECD document dating from 2002 is currently under revision.

1.3.4. Comparative analysis of composition

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

Comment:
The applicant concludes from the data that the nutritional composition of forage and grain derived from DAS maize is comparable to that of conventional maize, represented by non-GMO near isoline maize and non GMO commercial maize. **I agree with this conclusion.**

The applicant presents a lot of data as suggested in the OECD guidelines. However, taking into account the actual knowledge of maize the conclusion could be further confirmed by some additional information.

The important group of constituents in terms of oxidation, **tocotrienols** are not analysed. As already said they are important for the stabilization of the highly unsaturated maize oil. Tocopherols and tocotrienols receive a lot of attention in the actual approaches of nutritional value of foods.

No data are present in the field of **xanthophylls**. It is generally known het that maize is a good source of these constituents. In relation to their growing importance it would be useful to have information on the level of xanthophylls. I have no reason to doubt about their presence. However, due to their growing importance in human nutrition, particularly in eye health, for regions where maize is a staple food, these constituents contribute significantly to the nutritional value.

Note SBB & coordinator: The components analysed by applicants are based on the crop documents of the OECD. Tocotrienols and xanthophylls are not included in the list of components to be analysed for maize according to the OECD (https://www.oecd.org/env/ehs/biotrack/46815196.pdf). The OECD document dating from 2002 is currently under revision.

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:
I agree with the conclusion of the applicant.

1.3.7. Conclusion

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

Comment:
I agree with the overall conclusion of the applicant.

1.4. TOXICOLOGY

1.4.1. Testing of newly expressed proteins

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

Comment:

Statement: ***The potential toxicity of the Cry1Da2 protein was assessed by comparison of its sequence to the sequences in a toxin and general database (Annex 12). Bioinformatic analyses support the conclusions that the Cry1Da2 protein is unlikely to be a toxin.***
Verification: Annex 12 elaborates on the analyses to identify allergens (COMPARE 2021 database) and toxins (UniProtKB/Swiss-Prot, NCBI protein databases). No significant alerts were observed.
Conclusion: agree

Statement: ***Lability of the Cry1Da2 Protein in Sequential Digestibility Analysis with Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF).***
Verification In Annex 19, it was demonstrated that the Cry1Da2 protein migrating at ~68 kDa was digested within 0.5 minutes in SGF containing pepsin at pH ~ 1.2. On the SDS-PAGE gel, low molecular weight bands (~2-5 kDa) remained detectable in the Cry1Da2 protein samples for up to 60 minutes in SGF. The susceptibility of the low molecular weight SGF fragments (~2-5 kDa) of the Cry1Da2 protein was assessed in the sequential digestion (Annex 20). The Cry1Da2 protein was incubated for 1 minute in SGF and then incubated for 0, 0.5, 1, 2, 5, 10, 20, and 30 minutes in SIF containing pancreatin. Sequential pepsin and pancreatin digestion results indicated the low molecular weight bands (~2-5 kDa) observed in SGF digestion (Annex 19) were digested within 0.5 minutes during sequential SIF digestion.
Conclusion: agree

Statement: ***The results demonstrated that Cry1Da2 protein heat-treated at temperatures of 75 °C or higher was inactive against S. frugiperda when incorporated in an artificial insect diet.***

Verification: Annex 21 shows that "The results demonstrated that Cry1Da2 protein heat-treated for approximately 30 minutes at 75 °C and 95 °C was effectively inactive against *S. frugiperda* when incorporated in an artificial diet. Statistically significant decreases in protein activity were observed for Cry1Da2 protein heat-treated at temperatures of 75 °C, and 95 °C when compared to the unheated control."

Conclusion: agree

Statement: ***See section 1.5 for the results of the glycosylation analysis.***

Verification: Section 1.5 shows a scan of Annex 1 showing that Cry1Da2 is not glycosylated

Conclusion: agree

Statement: ***The Cry1Da2 protein derived from DAS1131 maize and the microbially derived Cry1Da2 protein is functional of the expected molecular weight, immunoreactivity, amino acid sequence, and showed a lack of glycosylation.***

Verification: The microbially derived Cry1Da2 protein contains two intended amino acid modifications relative to the DAS1131 maize-derived protein. Both the lysine at position 19 and the arginine at position 27 were changed to glutamine to avoid truncation and ensure intactness of the N-terminus during production of the test substance.

Information found in Section 1.2.1.3, Annex 1, Annex 2 , Annex 3, Annex 4 and Annex 5.

Section 1.2.1.3: The DAS1131 maize-derived Cry1Da2 protein migrated as two predominant bands: the upper Cry1Da2 protein band was consistent with the expected molecular weight of approximately 68 kDa and the molecular weight observed for the microbially derived Cry1Da2 protein. The lower band migrated at approximately 66 kDa. For the lower band of the DAS1131 maize-derived Cry1Da2, the nineteen amino acids of the N-terminus were not detected by LC-MS, indicating N-terminal truncation which was likely due to proteolysis by trypsin-like proteases in planta or during extraction and purification (Figure 1.2.1-4).

Annex 1: The Cry1Da2 protein derived from DAS1131 maize was characterized and had the expected molecular weight, immunoreactivity, amino acid sequence, and lack of glycosylation.

Annex 2: Purity and concentration were established for the microbially derived Cry1Da2 protein. The microbially derived Cry1Da2 protein had the expected molecular weight, immunoreactivity, amino acid sequence, and lack of glycosylation, and demonstrated bioactivity. The protein lot analyzed in this study is hereby considered characterized for use in regulatory studies.

Annex 3: Similar analysis and conclusions as in Annex 2, yet specifically for Lot TSN318947. The matching peptides was 93% for the latter as compared to 86% in Annex 2.

Annex 4: The Cry1Da2 protein was partially purified from DAS1131 maize and had the expected molecular weight and immunoreactivity. The concentration of the DAS1131 maize-derived Cry1Da2 protein was determined to be 1.04 mg Cry1Da2 protein/ml.

Annex 5: Based on the observations of this study, the microbially derived Cry1Da2 protein test substances (lot numbers TSN318947 and PCF-0056) demonstrate similar levels of bioactivity and overlapping confidence intervals. While the LC50 value was lower for the maize-derived protein, all test substances were biologically active in this study design. It is concluded that the TSN318947 and PCF-0056 Cry1Da2 test substances represent reasonable surrogates with respect to bioactivity for the DAS1131 maize-derived Cry1Da2 protein.

Conclusion: agree

Statement: ***The acute oral toxicity tolerant dose and the LD502 of Cry1Da2 protein was determined to be greater than 5000 mg/kg body weight.***

Verification: Annex 25 Based on the results of this study, oral (dietary) exposure of Cry1Da2 protein to Crl:CD1(ICR) mice at target exposure levels of 300 and 1000 mg/kg of bw/day for at least 28 consecutive days was well-tolerated at all target exposure levels with no test substance related mortality or adverse findings. The no-observed-effect level (NOEL) was 1000 mg/kg of bw/day equivalent to 879 and 1170 mg/kg of bw/day Cry1Da2 protein for males and females, respectively.

Conclusion: agree

Statement: ***Oral (dietary) exposure of Cry1Da2 protein to Crl:CD1(ICR) mice at target exposure levels of 300 and 1000 mg/kg of bw/day for at least 28 consecutive days was well-tolerated at all target exposure levels with no test substance related mortality or adverse findings. The no-observed-effect level (NOEL) was 1000 mg/kg of bw/day equivalent to 879 and 1170 mg/kg of bw/day Cry1Da2 protein for males and females, respectively.***

Verification: Annex 27 Reported information are fully in line with statement above.

Conclusion: agree

Statement: ***Taken together, the data from these assessments support the conclusion that the Cry1Da2 protein is unlikely to be a toxin to humans or animals.***

Verification: based on the elements evaluated above, but it is unclear why an acute toxicity study was provided since (EU) No 503/2013 states clearly in 1.4.1 “Acute toxicity testing of the newly expressed proteins of genetically modified plants is of little additional value for the risk assessment of the repeated human and animal consumption of genetically modified food and feed and shall not be provided as part of the studies performed under this point.”

Conclusion: agree

Statement: ***The potential toxicity of the DGT-28 EPSPS protein was assessed by comparison of its sequence to the sequences in a toxin and general database (Annex 12). Bioinformatic analyses support the conclusions that the DGT-28 EPSPS protein is unlikely to be a toxin.***

Verification: Annex 12

The allergen database used for the searches was the Comprehensive Protein Allergen Resource (COMPARE)2021 database (January 2021). None of the translated stop codon-bracketed reading frames in DAS1131 maize produced an eight contiguous amino acid match to an allergen. These data indicate that there is no allergenicity concern regarding the translated stop codon-bracketed reading frames in DAS1131 maize.

No alignments with an E-value ≤ 10-4 were returned between a translated stop codon-bracketed reading frame and any protein sequence in the internal toxin database. Eleven translated stop codon-bracketed reading frames produced alignments to protein sequences in the NCBI-nr protein database with E-values ≤ 10-4, none to toxins. None of these proteins is toxic to human and animals.

Bioinformatics evaluation of the DAS1131 maize insert did not generate biologically relevant amino acid sequence similarities to known allergens, toxins, or other proteins that would be harmful to humans or animals.

Conclusion: agree

Statement: ***It was demonstrated that the DGT-28 EPSPS protein migrating at ~45kDa was digested within 0.5 minutes in SGF containing pepsin at pH ~ 1.2.***

Verification: Annex 22 The DGT-28 EPSPS protein migrating at ~45 kDa was digested within 0.5 minutes in SGF as was evident in both the stained SDS-PAGE gel and western blot. Low molecular weight bands (~2 and ~5 kDa) on the SDS-PAGE gel remained detectable in the DGT-28 EPSPS protein samples for up to 60 minutes in SGF. As expected, the BSA control was digested in 1 minute and low molecular weight bands remained detectable at 60 minutes. The β-lactoglobulin control remained detectable after 60 minutes in SGF.

Annex 23 Sequential pepsin and pancreatin digestion results indicated the low molecular weight bands (~2 and ~5 kDa) observed in Annex 22 from SGF digestion were digested within 0.5 minutes during sequential SIF digestion (Figure 1.4-2).

Conclusion: agree

Statement: ***DGT-28 EPSPS protein was inactivated when heat-treated for 30-35 minutes at 50 °C and 75 °C. In addition, the DGT-28 EPSPS protein showed substantially reduced activity when heat-treated for the same length of time at 37 °C (33.4% activity compared to the unheated control). No reduction in activity was observed for DGT-28 EPSPS protein when heat-treated at 25 °C.***

Verification: Annex 24 confirms statement

Conclusion: agree

Statement: ***The results of the glycosylation analysis: section 1.5: Glycosylation was determined to be negative for the DAS1131 maize-derived and microbially derived DGT-28 EPSPS proteins (Figures 1.5-2).***

Verification: Annex 6 and Annex 7

Conclusion: agree

Statement: ***The acute oral toxicity tolerant dose and the LD50 of DGT-28 EPSPS protein was determined to be greater than 2000 mg/kg body weight.***

Verification: Annex 26; acute oral study (single dosing) at 2000 mg/kg (OECD 423) - no lethality, no clinical sings or impact on body weight (development). Tables 1 to 7 are lacking in the report (blank pages).

Conclusion: agree, but with comment on the quality (completeness) of the report

GMO Panel response: In relation to the missing tables, it is noted that the Annex 26 is an acute toxicity study report. As such, tables 1,2,3,4,5,6, and 7 are not required, as they contained data related to mean body weights, mean body weight gains, summary of clinical observations and mortality and incidences of gross observations.

Statement: ***The data from these assessments support the conclusion that the DGT-28 EPSPS protein is unlikely to be a toxin to humans or animals.***

Verification: The above-mentioned elements indeed support the conclusion about the safety of DGT-28 ESPS. However, it is unclear why an acute toxicity study was provided since (EU) No 503/2013 states clearly in 1.4.1 “Acute toxicity testing of the newly expressed proteins of genetically modified plants is of little additional value for the risk assessment of the repeated human and animal consumption of genetically modified food and feed and shall not be provided as part of the studies performed under this point.”. Moreover, no 28-day repeated dose study was conducted with DGT-28 EPSPS as required by (EU) No 503/2013, and this contrary to the Cry1Da2 protein in the same application and without justification. The application highlights the

fact that "*DGT-28 EPSPS expressed in DAS1131 is another iteration of this trait in a familiar crop. The mechanistic understanding and extensive experience with this trait provide information that can be used in a history of safe use evaluation (Conko et al., 2016).*" If a 28-day repeated dose study is not considered necessary, it would have been more transparent and consistent to address the information requirement of a 28-day study as stipulated by No 503/2013 specifically by elaborating a weight of evidence approach or read-across approach.

Conclusion: partially agree – unnecessary acute toxicity study and lacking information on repeated dose (28-day) toxicity.

GMO Panel response: The applicant presented additional information based on the sequence, structural and functional similarity between the DGT-28 EPSPS and other enzymes from class I and class II, which were considered by the GMO Panel. For details, please see section 3.5.1.2 and section 3.5.2.1 of the scientific opinion.

Summary of comments/questions:
Why acute toxicity studies with the 2 new proteins provided while not required by EU No 503/2013?
Why no 28-day repeated dose toxicity study or alternative explanation/justification (weight of summary/read-across) provided for DGT-28 EPSPS while required by EU No 503/2013?

Note SBB & coordinator: A reason to why acute toxicity studies are provided by the applicant is indeed not provided in the dossier. Often, although not required, available information is provided in a dossier as it provides extra evidence.

Most likely, the structural similarity (at domain level – see Griffin et al., 2021) of DGT28-EPSPS to EPSPS proteins already present in commercialised GM crops, and thus its 'history of safe use', is the underlying reason as to why no 28-day repeated dose toxicity study was provided. However, the scientific reason for not following the requirements of the Implementing Regulation could indeed have been more explicitly elaborated.

Comment 2:
Could the authors provide the exact protocol - exact keywords - to build their toxin database? What they write in Annex 12 is incomplete: "To produce the internal toxin database, the proteins in UniProtKB/Swiss-Prot are filtered for molecular function by keywords that could imply toxicity or adverse health effects (e.g., toxin, hemagglutinin, vasoactive, etc.)."

The digestibility analysis is performed at very acidic pH (1.2), but within the range of the EFSA (2017) recommendation. For Cry1Da2 and DGT28-EPSPS: the peptides persisting after digestion in SGF are large (> 9 amino acids). They seem to disappear when SGF is followed by SIF.

The legend in Figure 1.4.1 ("... SGF 10 minutes...") is different from that in Figure 1 in Annex 20 ("... SGF 1 minute..."): can the authors confirm the conditions under which the experiment was conducted?

Note SBB & coordinator: The information on how to build the toxin database is considered sufficient (given the etc.).

GMO Panel response: In relation to the discrepancy noted between the legend in figure 1.4.1 and that in Figure 1 in Annex 20 the applicant provided clarifications in the frame of ADR5 (21/02/2024). The sample description table in figure 1.4-1 of the technical dossier (Part II section 1.4_Toxicology) contains an error and should have stated a SGF of 1 minute as indicated in the text (p.3 of Part II).

1.4.2. Testing of new constituents other than proteins

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

1.4.3. Information on natural food and feed constituents

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

1.4.4. Testing of the whole genetically modified food or feed

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

Comment:
Are the rates of glyphosates used for the DAS1131 maize crop (Durango DMA 2.63 L/ha) equivalent to what is used by farmers under real conditions? It seems to me that this information is important in order to assess the difference between treated and untreated maize. This assessment must be done with maize crop grown in real glyphosate usage conditions.

Note SBB & coordinator: In Annex 28, reference is made to the report PHI-2020-018/002 which most likely contains more information on the crop maintenance practices. However, this report is lacking and is requested to verify whether the rates of glyphosate applied correspond to real conditions.

GMO Panel response: The GMO Panel thanks Belgium for the comment and confirms that the application rate has been verified and is in line with the recommendations of the manufacturer.

1.4.5. Conclusion of the toxicological assessment

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

Comment:

I agree with the overall conclusion

1.5. ALLERGENICITY

1.5.1. Assessment of allergenicity of the newly expressed protein

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

Comment:
Bioinformatics techniques show that the Cry protein under consideration has no similarities to existing allergens or toxins listed as such. The EFSA protocols are based solely on sequence identity with known allergens, and are therefore not able to identify new allergens that are clearly distinct from known allergens.
I draw attention to the fact that the literature on the potential allergenicity or toxicity of Cry proteins presents divergent results. The review article by Rubio-Infante & Moreno-Fierros (2016) is a critical and independent review of the literature on allergenicity and toxicity of Cry proteins. In the opinion of these authors "the term 'toxic' is not appropriate for defining the effects these toxins have on mammals", but they also argue for additional mammalian testing as knowledge is still limited.

The authors of the DAS1131 application write "Several Cry proteins have been deployed as safe and effective pest control agents in microbial Bt formulations for almost 40 years.". Several studies

point in this direction. But other studies have shown effects on mammals: see the review of Rubiante-Infante & Moreno-Fierros (2016). The authors should moderate their claim.

The bioinformatics study of the allergenicity of both proteins was carried out correctly (according to the EFSA protocols) and did not show any identity with known allergens.

Digestibility test: see comment in section 1.4.1.

I agree with the analysis of the allergenicity of potential ORFs.

Note SBB: Proposals for textual changes are not considered at this stage of the evaluation process.

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. Conclusion of the allergenicity assessment

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

1.6. NUTRITIONAL ASSESSMENT

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

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

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

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. GENERAL APPROACH OF THE ERA

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

5.2. SPECIFIC AREAS OF RISK

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

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

5.2.2. Plant to micro-organisms gene transfer

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

5.2.3. Interactions of the GM plant with target organisms

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

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

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

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

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

5.2.6. Effects on biogeochemical processes

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

5.2.7. Effects on human and animal health

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

5.2.8. 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

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- Rubio-Infante N, Moreno-Fierros L. An overview of the safety and biological effects of Bacillus thuringiensis Cry toxins in mammals. J Appl Toxicol. 2016;36:630-48.

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

PART I - GENERAL COMMENTS

Comment:

The use of glyphosate in Europe is widely debated, with currently an authorisation that has been extended until the end of 2023. At the same time, an opinion is being sought on the import of this transgenic maize DAS1131 which is glyphosate resistant - and therefore grown in the presence of glyphosate. It is not for me to assess the environmental for the place of cultivation, if it is outside Europe. But isn't it schizophrenic to import glyphosate-resistant maize grown in the presence of glyphosate and at the same time to question the use of glyphosate in crops in Europe?

PART II - SCIENTIFIC INFORMATION

1.3.4. Comparative analysis of composition

Comment:
The comparative analysis focuses on constituents mentioned in het OECD guidelines. There is no information about the presence of particular toxicants, that could be relevant to maize: mycotoxins and heavy metals.

Mycotoxins are a point of attention in safety considerations of maize. Is there any modification in the resistance to mould infection and potential mycotoxin formation?

Heavy metals are present in cereals and may be at the origin of concern about safety issues. This is, for instance, the case for rice, as particular rice varieties are known to take up heavy metals like arsenic from the environment. Is there any reason to consider this phenomenon for the newly developed maize?

1.4.5. Conclusion of the toxicological assessment

Comment:
The genetic construct gives maize resistance to glyphosate. Does the plant accumulate the sprayed glyphosate in this case?
Studies on glyphosate-resistant soybeans show higher glyphosate residues in the harvested GM plants than in non GM plants (Bøhn et al. 2014, Bøhn et al. 2019). Other studies show the potential toxicity of glyphosate (Myers et al. 2016), and the World Health Organization's International Agency for Research on Cancer re-classified glyphosate as "probably carcinogenic to humans" (Guyton et al. 2015) (although the US EPA reached a different conclusion about the possible carcinogenicity of glyphosate – see Benbrook 2019 for an analysis of this).

In this context, measuring glyphosate residues in the GM plants seems to me relevant in a toxicological analysis. This is not done in the toxicology assessment for DAS1131.