Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on application EFSA-GMO-DE-2016-137 (genetically modified maize GA21 x T25) from Syngenta under Regulation (EC) No. 1829/2003

21 March 2023
Ref. SC/1510/BAC/2023_0271

Context

Application EFSA-GMO-DE-2016-137 was submitted by Syngenta for the marketing of genetically modified (GM) maize GA21 x T25 (Unique Identifier MON-ØØØ21-9 x ACS-ZMØØ3-2), for food and feed uses, import and processing (excluding cultivation) within the European Union, within the framework of Regulation (EC) No. 1829/2003[1].

The two-event stack, maize GA21 x T25, was obtained by conventional crossing (no new genetic modification involved) of the corresponding single events:
- GA21, expressing the mEPSPS protein that confers tolerance to glyphosate-containing herbicides;
- T25, expressing the PAT protein for tolerance to herbicide products containing glufosinate ammonium;

The application was validated by EFSA on 23 February 2017. A formal three-month consultation period of the Member States was started, lasting until 24 May 2017, 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). Nine 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 19 May 2017.

The opinion of the EFSA Scientific Panel on GMOs was published on 27 January 2023 (EFSA Journal 2023;21(1):7729[2]), together with the responses from the EFSA GMO Panel to comments submitted by the Member States during the three-month consultation period. On 23 February 2023 these two documents were forwarded to the Belgian experts. They were invited to give comments and to react if needed.

In delivering the present advice the BAC considered in particular the following information:
- The comments formulated by the experts on application EFSA-GMO-DE-2016-137;
- The opinion of EFSA;
- The advices already adopted by the BAC on the single events. The conclusions of the BAC for the most recent applications for the single events were as follows:

Event	Application number	BAC advice	Conclusions
GA21	EFSA-GMO-RX-005	BAC/2018/0058	No major risks for human and animal health or concerning the environment were identified.
T25	EFSA-GMO-NL-2007-46 and RX-T25	BAC/2014/0329	Unlikely to pose any risk to human and animal health or the European environment.

[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://www.efsa.europa.eu/en/efsajournal/pub/7729

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Scientific evaluation

1. Molecular characterisation

With regard to the molecular characterisation, the Biosafety Advisory Council is of the opinion that the information provided is sufficient and does not raise safety concerns.

2. Assessment of food/feed safety and nutritional value

2.1. Assessment of compositional analysis

Taking into account the previous assessment of the single events and the new data on compositional analysis provided by the applicant for the two-stacked event, the Biosafety Advisory Council agrees with the GMO panel of EFSA that the compositional data of GM maize GA21 x T25, 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 mEPSPS and PAT proteins in the context of previous applications, and no safety concerns with respect to toxicity 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 presence of the newly expressed proteins in the stacked event does not raise toxicological concerns.

2.3. Assessment of allergenicity

The Biosafety Advisory Council has evaluated the safety of the newly produced 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 stacked 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 maize GA21 x T25-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

[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/

wild relative in the EU. Therefore, the Biosafety Advisory Council is of the opinion that it is unlikely that the accidental release of maize GA21 x T25 (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 GA21 x T25 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 two single events, the Biosafety Advisory Council:

1) Agrees with the GMO panel of EFSA that the potential environmental release of maize GA21 x T25 is unlikely to pose any threat to human and animal health and 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;

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 EFSA-GMO-DE-2016-137 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: Eddy Decuypere (KUL), Jacques Dommes (ULg), Patrick du Jardin (ULg-Gembloux), Leo Fiems (ILVO), Johan Grooten (UGent), André Huyghebaert (UGent), Peter Smet (Consultant), Frank Van Breusegem (UGent), Jan Van Doorsselaere (KATHO).
SBB: Katia Pauwels, Fanny Coppens

Application: EFSA-GMO-DE-2016-137
Applicant: Syngenta
GMO: Maize GA21 x T25
Validation of dossier by EFSA: 23 February 2017

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

GENERAL COMMENTS

Comment 1
No questions

Comment 2
No comment, adequate information was provided

Comment 3
From a biological point of view GM GA21 x T25 maize may be as safe for human and animal health and the environment as conventional maize based on the results of the compositional analysis and the toxicological and allergenicity assessment.
However, the risk assessment should also take the potential for accumulation of residues and metabolites of the herbicides into account, against which GA21 x T25 maize is tolerant. Furthermore, a combined margin of exposure is lacking.

***Comment SBB**: The safety of glyphosate-based herbicides is not within the remit of the BAC.*

Comment 4
Stacked GA21 x T25 will be further referred to as 137 maize.

Comment 5
Part VII Summary Info: folder is empty ? only a .db file present. I could not find several appendices or references that contain info that is necessary to comprehensively assess the application (see below)

Comment SBB: It is correct that the folder 'Part VII' erroneously was left empty on the extranet for the experts. However, the content of this folder is limited to a summary of the dossier (pdf-file), which was integrally attached to the e-mail sent on 20 March 2017 (request for expertise).

A. HAZARD IDENTIFICATION AND CHARACTERISATION

A.1. INFORMATION RELATED TO THE RECIPIENT OR (WHERE APPROPRIATE) THE PARENTAL PLANT

Comment 1
No comment, adequate information was provided

Comment 2
No comments

Comment 3
No comments

A.2. MOLECULAR CHARACTERISATION

A.2.1. INFORMATION RELATING TO THE GENETIC MODIFICATION Including:

- Description of the methods used for the genetic modification
- Source and characterization of nucleic acid used for transformation
- Nature and source of vector(s) used

Comment 1

GA21xT25 maize is produced by conventional crossing of GA21 expressing mepsps gene, providing tolerance to glyphosate, and T25 expressing pat-gene, providing tolerance to glufosinate herbicide.

Comment 2

No comment, adequate information was provided

Comment 3

- I assume that detailed info on is presented in the EFSA-GMO-NL-2007-46 & 2005-19 files. However I could not find these files. It would be interesting to know whether for T25, the plasmid was linearized and how.

Comment SBB: For T25 (EFSA-GMO-NL-2005-19), the plasmid pUC/Ac, also named p35S/Ac plasmid, was used for transformation and PCR data indicated that the vector pUC/Ac DNA sequence has been integrated from bp 3814 to 3555 into the corn genome. PEG mediated protoplast transformation was carried out for intended insertion of P35S-pat-T35S cassette into the plant genome. No further information was provided with respect to linearization of the plasmid.

- What is meant with “The intended region of insertion for T25 is shown in…” It is more useful to how the adjacent genomic region and the plasmid is actually integrated. Figure 1.2-3 is not understandable without a legend. What is the “deleted” part et cetera.

Comment SBB: On pg 31 it is further specified that ‘*Further analysis of the event T25 revealed that the insert consists of the P35S-pat-T35S expression cassette and at the 3' end of the insert a duplication of an internal fragment similar to part of the P35S promoter, linked to a fragment of the bla gene. The duplicated P35S-like promoter fragment contains the sequences from bp 80 to bp 433 of P35S and the bla gene fragment contains the sequence from bp 196 until bp 861 of the bla gene. The first 5 bp of the bla gene (containing the transcription initiation codon ATG) are inserted at the 5' end of the insert* ‘. This information also corresponds to what has been described in EFSA-GMO-NL-2007-46, Part I: Section D).

- Indication of the EcoRI restriction site that generates the 1,3kb insert fragment for GA21 would be informative on the map.
-

Comment SBB: a EcoRI restriction site that generates the 1,3kb insert fragment correspond to the chimeric *pat* gene cassette that could be isolated as a 1.3 kb *Eco*R1 fragment for T25 (and not GA 21).

- In the map, the actin promotor, intron and exon are indicated separately (also their size), while in the table they are called “the promotor complex” .This is confusing.
- How where the adjacent regions obtained. What is their length ?

Comment 4
No comment

A.2.2. INFORMATION RELATING TO THE GM PLANT Including:

- Description of the trait(s) and characteristics which have been introduced or modified
- Information on the sequences actually inserted or deleted
- Information on the expression of the insert
- Genetic stability of the inserted/modified sequence and phenotypic stability of the GM plant

Comment 1
Same structure of the insert in stacked event as in GA21 and T25 maize, same level of expression. Stable traits; no potential for production of new toxins or allergens and no unintended changes or modifications were identified in the stacked event.

Comment 2
The applicant claimed that "no nucleotide changes were identified in the GA21 insert and flanking sequences present in GA21 x T25 maize genomic DNA compared to the previously determined GA21 insert and flanking sequences reported in GA21 maize". Reference is made to appendices 1.2-2 and 1.2-3. I did not find these appendices in the dossier. Therefore I was not able to check this claim. However Southern blot analyses (appendix 1.2-1) as well as expression analyses and phenotypic characteristics show that inserts of the single events GA21 and T25 were kept intact in the GA21 x T25 stacked maize.

Comment SBB: According to App B – Table 1 List of appendices, the appendices 1.2-2 and 1.2 -3 were provided as new document (confidential information), however these appendices were lacking from the dossier as loaded on EFSA extranet and could therefore not be transferred to the SBB extranet.

Comment 3
Comment A:
In the section 1.2.2.2. Information on the sequences actually inserted, the applicant refers to the "parental line" for the sequence comparison with the stack: "*Sequence information on T25 maize found in GA21 x T25 maize is provided in Appendix 1.2- 4 (and related Appendices therein), which showed a 100% identity alignment of the T25 transgenic locus sequence in GA21 x T25 with the T25 transgenic locus of the parental line."*
I wonder whether the "parental line" to which it is referred to corresponds well with the single event previously risk assessed. When trying to know more about this, it came out that the Appendix 1.2-2 (and related Appendices therein) is missing in the web folder of SBB. Can you check that?
This raises a general question of interpretation of the Article 1.2.2.4 of the implementing regulation EU No 503/2013 :
The applicant shall provide information:
(…)
(b) in case of stacked transformation events, to establish that each of the transformation events stacked in the plant has the same molecular properties and characteristics as in the plants with the single transformation events"
It is implicit that the single event to be compared with is the one previously risk assessed and for which a detailed analysis of the sequence has been made. Would it be acceptable to use as sequence comparator the parental lines used for the commercial production of the stack?

Comment SBB: According to App B – Table 1 List of appendices, the appendices 1.2-2 and 1.2 -3 were provided as new document (confidential information), however these appendices were lacking from the dossier as loaded on EFSA extranet and could therefore not be transferred to the SBB extranet.

Comment B:

In the up-to-date bioinformatic search for homology with toxins and allergens of new ORFS created by the genetic transformation, the *E*-value set as a threshold of significance by the applicant is $1x10^{-5}$ (main text p. 83 and report 1.2-11 on page 8). This threshold of significance is used after using a higher *E* value for reporting alignments (= 10). Quoting the main text on page 83:

The E-values of < 1 × 10-5 were used as a threshold of significance for sequences requiring further inspection of the quality and relevance of the similarity; for alignments occurring below the initial E-value of 10.

Could the applicant provide a justification of why the *E* value of 10^{-5} was chosen?

Feedback from the EFSA GMO Panel: The applicant submitted updated bioinformatic analyses in Clock17 (18/11/2022). For the bioinformatic analysis for toxicity, the applicant used the E-value threshold of 10 to retrieve all possible alignments while considering the alignments significant only with E-value=10E-5. Overall, considering that no specific criteria are set by EFSA for this analysis, the approach was considered acceptable by the GMO Panel. Nevertheless, overall the analyses did not identify biologically significant hits to toxins.

Comment C:

The applicant points out two significant differences between the protein levels (mEPSPS and PAT) when comparing the stack and the corresponding single events (main dossier p.41 and quoted report 1.2-14). This raises no concern as these differences are noted in specific tissues at specific stages only (leaves and whole plants at the V6 stage), which do not correspond to imported materials and can be considered as out of scope. Furthermore, these differences are not sufficient *per se* to suggest interaction between the events combined in the stack.

Comment 4

The amounts of the proteins PAT and mEPSPS in the stacked event are comparable to those in the parental lines.

Comment 5

I could not find the Appendices 1.2-1,1.2-2, 1.2-3, and 1.2-4 (mentioned on p.31)

Comment SBB (same comment as under A.2.2.): According to App B – Table 1 List of appendices, the appendices 1.2-2 and 1.2 -3 were provided as new document (confidential information), however these appendices were lacking from the dossier as loaded on EFSA extranet and could therefore not be transferred to the SBB extranet.

Comment 6

No comments

A.3. COMPARATIVE ASSESSMENT

A.3.1. CRITERIA FOR THE SELECTION OF COMPARATOR(S)

Comment 1
No questions

Comment 2
No comments

Comment 3
137 maize is compared with the conventional counterpart.
No remarks.

A.3.2. FIELD TRIALS: EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

Comment 1
No questions

Comment 2
The experimental design included a treatment with the combined application of glyphosate and glufosinate, against which GA21 x T25 maize is tolerant. This is in agreement with the guidelines of EFSA (2010). Consequently, it is evident to take this factor into account for the comparative analysis, including the residue concentrations of glyphosate and glufosinate in GA21 x T25 maize.

Comment SBB: *The assessment of the safety of glyphosate and glufosinate and metabolites are not within the remits of the BAC.*

Comment 3
No remarks

A.3.3. COMPOSITIONAL ANALYSIS

Comment 1

Why alpha-tocopherol (statistically significant different between GM and control) has been measured and not total tocopherol, or beta, gamma, delta tocopherol who are more abundant in plant tissue than alpha (this is rather in animal tissues); see p60 and p64, fig 1.3.6 (p63))

I do not understand this reasoning that no further assessment is needed for the statistical differences between GM and non-GM conventional counterpart for oleic-acid and copper in grain “because there are no interactions between test material and site”?

I can agree that there is no reason of concern however because there is equivalence to the reference lines in all cases

Comment coordinator: So, no reason to send in comments about this.

Comment 2

Some compounds of GA21 x T25 maize were significantly different from conventional maize: fat, starch, ash, phosphorus, potassium, ... It may be recognized that if a compositional variation is detected, this should not be inferred as representing a de facto hazard. About half of the stack ranges with values outside of the prediction intervals occur among the minerals, which are not metabolized by the plant but are influenced by environmental conditions as soil type and fertilization (Kramer et al., 2016). Furthermore, a recent study reported that the heterogeneity is rising in maize since 2000, both between and within fields (Lobell and Azzari, 2017).
No residue concentrations were given for glyphosate and glufosinate in case of GA21 x T25 maize treated with these herbicides. So, what is the relevance of including treatments with these herbicides in the experimental design if part of the results is omitted? Recombinant GA21 x T25 maize exhibiting herbicide tolerance may indirectly result in the potential for accumulation of residues, altered metabolites of such residues, toxic metabolites, contaminants, or other substances that may be relevant to human health. The risk assessment should take this potential for accumulation into account (FAO, 2009). Therefore, it is desirable to analyse residues and metabolites of glyphosate and glufosinate in GA21 x T25 maize.

Comment SBB: The assessment of the safety of glyphosate and glufosinate and metabolites are not within the remits of the BAC.
Comment coordinator: The treatments with herbicides are included because you want to take into account possible effects of herbicide treatments on the composition of the maize, excluding the herbicides and their metabolites. These latter components are part of the herbicide and MRL assessments.

Comment 3
I will not discuss forage and limit my comments to maize grain.

Comments on the selection of compounds for analysis:
- the OECD guidelines of 2002 are followed
- 15 years later knowledge about the composition of maize and the role of maize constituents in human nutrition changed consistently; a revision of the guidelines is suggested
- in proximate analysis no information is available on dietary fiber, only acid detergent and neutral detergent fiber, data from animal nutrition, are available; carbohydrates are given as a group with no differentiation with the exception of starch,
- minerals are studied in detail; no information is available on the presence of undersirable trace elements or the potential specific uptake of heavy metals from the soil,
- relevant vitamins are well covered,
- maize germ oil is highly unsaturated; the oil is protected against oxidation by the presence of tocopherols and tocotrienols; the only compound studied is α-tocopherol or vitamine E; the most potent antioxidative constituents are not included,
- provitamin A or β-carotene is studied; there is no information on other compounds such as xantophyll and lutein
- amino acids and fatty acids are studied in great detail,
- no comment on the secondary metabolites and anti-nutrients,

As a general comment the 2002 OECD guidelines have to be adapted to the actual state of the art of nutritional composition of maize.

Feedback from the EFSA GMO Panel: The GMO Panel took note of the comment.

Data from the analyses are statistically evaluated and graphically represented. They are classified according to the Efsa scheme of equivalence or non-equivalence.
In most cases no statistical differences are found. For a few compounds differences were identified but the data are within the natural variations of maize.

There is the observation that for this stacked 137 maize statistical differences are only observed in a few cases. This was not the case for other applications for GM-maize where more differences, but within biological variability, were identified.
A further study could clarify this matter.

The applicant concludes that the nutritional and antinutritional components of 137 maize are equivalent to the non transgenic reference lines. Levels of components are within the normal ranges of conventional maize.

I agree with this conclusion.

Comment coordinator: In regards the suggestion to further clarify the fewer cases of statistical differences for this stacked maize, why would it be relevant to look at why in this particular case there are less differences than seen in other cases?

Comment 4
Secondary metabolites and antinutrients:
Either no significant difference from the control or equivalent to the reference lines.

A.3.4. AGRONOMIC AND PHENOTYPIC CHARACTERISTICS

Comment 1
No questions

Comment 2
No comments

Comment 3
No comments on the results with the exception that some attention is given to diseases but no information is available on the presence of metabolites due to mould infection.
Maize is known to be one of the most sensitive grains for the presence of mycotoxins.

As a general comment it is suggested that food safety aspects like the potential presence of mycotoxins should be included in the overall evaluation of GM maize

Comment SBB: Concerning the comment above on mycotoxins, it is important to remind that the objective of the compositional analysis is to compare the genetically modified plant and its conventional counterpart in order to identify possible unintended effects resulting from the genetic modification.

A.3.5. EFFECTS OF PROCESSING

Comment 1
No questions

Comment 2
No particular effects are to be expected.

A.4. TOXICOLOGICAL ASSESSMENT

A.4.1. METHODOLOGY USED FOR TOXICITY TESTS

Comment 1
No questions

A.4.2. ASSESSMENT OF NEWLY EXPRESSED PROTEINS including:

- Molecular and biochemical characterisation of the newly expressed proteins
- Up-to-date bioinformatic search for homology
- Information on the stability of the protein under the relevant processing and storage conditions for the food and feed derived from the GM plant
- Data concerning the resistance of the newly expressed protein to proteolytic enzymes
- Repeated dose toxicity studies using laboratory animals

Comment 1
No questions in view of previous assessment of the molecular and biochemical characterization of mEPSPS and PAT proteins.

Comment 2
Evaluated. No safety concern.

Comment 3
No comments – these analyses were performed in the single events previously assessed, with an update in the bioinformatic analyses

Comment 4
The chance that the new proteins of T25 and GA21 maize (mEPSPS and PAT) in GA21 x T25 maize will pose serious risks for toxicity is negligible, based on the biochemical characterization of the newly expressed protein, the bioinformatics analysis that uses sequence searches to identify any similarities to toxins and anti-nutrients, inactivation of new proteins during heat processing and the in-vitro protein stability.
EFSA (2013 and 2015a) did not identify safety concerns in the case of T25 and GA21 maize, respectively. Therefore, no safety problems are expected in the case of GA21 x T25 maize, where the mEPSPS and PAT proteins are identical to the mEPSPS protein produced in GA21 maize and the PAT protein produced in T25 maize. We assume that there is no biological pathway in which the newly-inserted genes would directly or indirectly interact with safety (Kok et al., 2014; Zdziarski et al., 2014). There is also no plausible or testable hypothesis for an interaction of the new proteins in GA21 x T25 maize (Steiner et al., 2013). WHO (1995) stated that, when two plants that are substantially equivalent to conventional varieties are crossed by conventional breeding, the stacked event is expected to be substantially equivalent to the single events.
The main text and the appendix dealing with the rat study did not mention an adverse effect in the 13-week study, conducted with Han Wistar rats. However, deformed new-born pigs were reported when

glyphosate tolerant GM soy was used (Sørensen et al., 2014), so that some caution may be warranted. An animal study with respect to the reproductive and developmental toxicity of GA21 x T25 maize was carried out (Technical Dossier, P.87).

Comment 4
Previous test showed rapid degradation in both SGF and SIF. Previous test showed no signs of acute toxicity.

No biologically relevant identities were found with any toxic proteins. (2016)

A.4.3. ASSESSMENT OF NEW CONSTITUENTS OTHER THAN PROTEINS

Comment 1
No new constituents other than proteins

A.4.4. ASSESSMENT OF ALTERED LEVELS OF FOOD AND FEED CONSTITUENTS

Comment 1
No questions

A.4.5. ASSESSMENT OF THE WHOLE FOOD AND/OR FEED DERIVED FROM GM PLANTS

Comment 1
No questions

Comment 2
90-Day rat study.
There were no toxicological effects noted on body weight, food consumption, clinical condition (including neurotoxicity assessments), ophthalmoscopy, haematology, coagulation, blood chemistry or macroscopic and microscopic pathology at inclusion levels up to and including 41.5%.

A.5. ALLERGENICITY ASSESSMENT

A.5.1. ASSESSMENT OF ALLERGENICITY OF THE NEWLY EXPRESSED PROTEIN including:
- Amino acid sequence homology comparison between the newly expressed protein and known allergens using a comprehensive database
- Specific serum screening
- Pepsin resistance and in vitro digestibility tests
- Additional tests

Comment 1
No questions

Comment 2
Evaluated. No safety concern.

Comment 3
No comments – these analyses were performed in the single events previously assessed, with an update in the bioinformatic analyses.

Comment 4
The risk analysis of the individual genetic traits has been updated from the previous applications using the 2016 FARRP allergen database. From this (bioinformatics) analysis novel risks for allergenicity of the introduced double-mutated 5-enolpyruvylshikimate-3-phosphate synthase (mEPSPS) and phosphinothricin acetyltransferase (PAT) proteins did not emerge. Furthermore, I agree with the applicant that the stacking of both traits in this GA21 x T25 maize is unlikely to result in mutual interactions that may increase the risk for allergenicity.

I have no further comments.

A.5.2. ASSESSMENT OF ALLERGENICITY OF THE WHOLE GM PLANT

Comment 1
No questions

Comment 2
It is assumed that GA21 x T25 maize has no greater allergenic potential compared to the parent plants GA21 maize and T25 maize, and conventional commercial maize varieties, and that it does not pose a serious allergenic risk.

Comment 3
The reviewer particularly appreciates that a 90-day feeding study with GA21 x T25 maize has been performed in the frame of the toxicological assessment. The absence here of a phenotype along with the absence of biologically relevant differences between the hybrid GA21 x T25 maize and its conventional counterparts in the comparative compositional assessment and more theoretical arguments such as the independent biological roles of both enzymes in different metabolic pathways indeed do not point towards an increased risk of allergenicity of the whole GM plant.

From looking at the year of EFSA approval, I guess commercial exploitation of either parent strain is still quite recent and therefore no health monitoring reports on the individual GMO's are yet available?

I have no further comments.

A.5.3. ADJUVANTICITY

Comment 1
No questions

Comment 2
No comment

A.6. NUTRITIONAL ASSESSMENT

A.6.1. NUTRITIONAL ASSESSMENT OF FOOD DERIVED FROM GM PLANTS

Comment 1
No questions

A.6.2. NUTRITIONAL ASSESSMENT OF FEED DERIVED FROM GM PLANTS

Comment 1
No questions

Comment 2
Some compounds of GA21 x T25 maize were significantly different from conventional maize. The applicant stated that animal feed products from GA21 x T25 maize are substantially equivalent to feed derived from commercial maize (P.73 of the Technical Dossier): this is not completely in agreement with comment on compositional analysis (A.3.3), where it was mentioned that some compounds of GA21 x T25 maize were significantly different from conventional maize.
Therefore, a general surveillance should be used to evaluate if the observed difference poses a risk to food and feed safety or the environment.

Comment coordinator: General surveillance is a standard requirement for each and every GM food crop that is introduced onto the European market.

B. EXPOSURE ASSESSMENT - ANTICIPATED INTAKE/EXTENT OF USE

Comment 1
No questions

Comment 2
The Technical Dossier (P.97) only refers to the margins of exposure (MOE) for PAT protein. Why did the applicant not calculate a combined MOE for the mEPSPS and PAT? Furthermore, I miss an extended combined MOE, based on the 2 new proteins (mEPSPS and PAT) and the 2 herbicides (glyphosate, glufosinate), as proposed by Wilkinson et al. (2000), because of the risk of these herbicides with regard to human health.

Comment SBB: The safety of glyphosate-based herbicides is not within the remit of the BAC.

Feedback from the EFSA GMO Panel: The GMO Panel thanks Austria (*sic*) and took note of the consideration, although did not follow a MoE approach.

C. RISK CHARACTERISATION

Comment 1
No comments

D. POST MARKET MONITORING (PMM) OF FOOD AND FEED DERIVED FROM GM PLANTS

Comment 1
No questions

E. ENVIRONMENTAL RISK ASSESSMENT

E.1. INTRODUCTION

Comment 1
No questions

Comment 2
No comment, adequate information was provided

E.2. GENERAL APPROACH OF THE ERA

Comment 1
No questions

Comment 2
No comment, adequate information was provided

E.3. SPECIFIC AREAS OF RISK

As stated in the EFSA guidance on the environmental risk assessment of genetically modified plants (EFSA Journal 2010, 8(11):1879) the objective of the ERA is on a case-by-case basis to identify and evaluate potential adverse effects of the GM plant, direct and indirect, immediate or delayed (including cumulative long-term effects) on the receiving environment(s) where the GM plant will be released. For each specific risk the ERA consists of the six steps described in Directive 2001/18/EC:
1. Problem formulation including hazard identification,
2. Hazard characterisation,
3. Exposure characterisation,
4. Risk characterisation,
5. Risk management strategies,
6. Overall risk evaluation and conclusions.

E.3.1. PERSISTENCE AND INVASIVENESS INCLUDING PLANT-TO-PLANT GENE FLOW

Comment 1
No questions

Comment 2
No safety concern

Comment 3
No comments

E.3.2. PLANT TO MICRO-ORGANISMS GENE TRANSFER

Comment 1
No questions

Comment 2
No safety concern

Comment 3
No comments

E.3.3. INTERACTION BETWEEN THE GM PLANT AND TARGET ORGANISMS

Comment 1
Not relevant

Comment 2
No safety concern

E.3.4. INTERACTION BETWEEN THE GM PLANT AND NON-TARGET ORGANISMS (NTOS)

Comment 1
No questions

Comment 2
No safety concern

E.3.5. IMPACTS OF SPECIFIC CULTIVATION AND MANAGEMENT AND HARVESTING TECHNIQUES

Comment 1
Not applicable

Comment 2
Not relevant

Comment 3
GA21 x T25 maize is tolerant against glyphosate and glufosinate, which may result in an increased application of these herbicides, which may not prevent herbicide resistance on the long run (Evans et al., 2015). So, the problem is not the genetic modification in itself, but rather the management and the governance of this innovation with regard to the use of some herbicides against which as GA21 x T25 maize is tolerant, and that may pose some risk for human health.

E.3.6. EFFECTS ON BIOGEOCHEMICAL PROCESSES

Comment 1
Not relevant

Comment 2
No relevant

E.3.7. EFFECTS ON HUMAN AND ANIMAL HEALTH

Comment 1

No questions

Comment 2
No safety concern

Comment 3
In the case of genetically modified herbicide-tolerant crops, such as GA21 x T25 maize, the effect of the genetic modification cannot be isolated from the effect of the herbicides. High residue concentrations of glyphosate in GA21 x T25 maize grain and forage may inhibit rumen digestion in ruminants (Reuter et al., 2007). Furthermore, glyphosate has been detected in the urine of dairy cows (Krüger et al., 2013). Glyphosate has been detected in the urine of sows and in the tissue of deformed new-born pigs, showing that glyphosate is absorbed, circulates in the body and is finally deposited (Krüger et al., 2014; Sørensen et al., 2014). The latter authors concluded that that glyphosate is rather a risk factor than the GM crop itself.
Furthermore, human health concerns with regard to the use of glyphosate have been reported (Mensah et al., 2015). GA21 x T25 maize may indirectly result in the potential for accumulation of residues and metabolites that may be relevant to human health. According to FAO (2009) the risk assessment should take this potential for accumulation into account.

Comment coordinator: This is information that should be taken into account when re-evaluating glyphosate.
Comment SBB: The safety of glyphosate-based herbicides is not within the remit of the BAC.

E.3.8. OVERALL RISK EVALUATION AND CONCLUSIONS

Comment 1
No questions

Comment 2
I agree with these general conclusions

Comment 3
No comments

Comment 4
Weber et al. (2012) reported that there is no readily identifiable biological reason why genomic changes occurring in the breeding of a GM stack would be different in nature, scale, or frequency from those taking place in conventional crops or in GM crops with a single event. Pilacinski et al. (2011) concluded that combined GM event plants, produced through conventional breeding, can be considered to be safe, given the expected safety of the parent plants. Therefore, we expect no detrimental effect of the new proteins in GA21 x T25 maize on the nutritive value and animal and human health.
Because high doses of glyphosate may be toxic (Williams et al., 2016), the application doses during cultivation should be carefully respected. Even if GA21 x T25 maize is not intended for cultivation in the EU, the application high doses of glyphosate elsewhere may result in the presence of residues. Import of GA21 x T25 maize containing glyphosate residues may be detrimental for the health of the EU consumer.

Comment SBB: The safety of glyphosate-based herbicides is not within the remit of the BAC.

E.4. Post Market Environmental Monitoring plan

E.4.1. Interplay between environmental risk assessment and monitoring

Comment 1
No questions

Comment 2
No comments

E.4.2. Case-specific GM plant monitoring

Comment 1
No questions

Comment 2
No comments

E.4.3. General surveillance for unanticipated adverse effects

Comment 1
No questions

Comment 2
No comments

Comment 3
Because of some compounds of GA21 x T25 maize were significantly different from conventional maize (See A.3.3), and because of unexpected rearrangements of the integration site and inserted DNA have been identified in maize events T25 and GA21 (Collonnier et al., 2003), the general surveillance should check whether unexpected, adverse effects could occur. Furthermore, some vigilance is necessary until the effect of glyphosate on health in clarified.

Comment coordinator: General surveillance is a standard requirement.

E.4.4. Reporting the results of monitoring

Comment 1

Since the scope of this application is the authorization of GA21xT25 maize for import, processing, feed and food use in EU, but not for cultivation, the exposure to the environment will be limited to unintended release e.g. during unloading, spoilage etc. The environmental monitoring plan and monitoring methodology are well described and sufficiently detailed.

Comment 2
No comments

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