Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on application EFSA-GMO-NL-2013-113 (genetically modified maize MON 89034 x 1507 x MON 88017 x 59122 x DAS-40278-9) from Dow Agrosciences under Regulation (EC) No. 1829/2003

Adopted on 29 January 2019
Ref. SC/1510/BAC/2019_0101

Context

Application EFSA-GMO-NL-2013-113 was submitted by Dow Agrosciences for the marketing of genetically modified (GM) maize MON 89034 x 1507 x MON 88017 x 59122 x DAS-40278-9 (Unique Identifier MON-89Ø34-3 x DAS-Ø15Ø7-1 x MON-88Ø17-3 x DAS-59122-7 x DAS-4Ø278-9), and all its subcombinations independently of their origin, 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 five-event stack maize MON 89034 x 1507 x MON 88017 x 59122 x DAS-40278-9 was obtained by conventional crossing (no new genetic modification involved) of the corresponding single events:
- MON 89034, expressing the Cry1A.105 and Cry2Ab2 proteins for resistance to certain lepidopteran pests;
- 1507, expressing Cry1F protein that confers resistance to certain lepidopteran pests, and the PAT protein that confers tolerance to herbicide products containing glufosinate ammonium;
- MON 88017, expressing the Cry3Bb1 protein for protection against corn rootworm larvae and CP4-EPSPS for tolerance to glyphosate.
- 59122, expressing the Cry34Ab1, Cry35Ab1 proteins for protection against corn rootworm larvae and PAT protein for tolerance to glufosinate herbicides.
- DAS-40278-9, expressing the AAD-1 protein for tolerance to 2,4-D and AOPP herbicides.

The application was validated by EFSA on 2 October 2014. A formal three-month consultation period of the Member States was started on 7 June 2017, lasting until 7 September 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). Six 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.

The opinion of the EFSA Scientific Panel on GMOs was published on 14 January 2019 (EFSA Journal 2019;17(1):5521[2]), together with the responses from the EFSA GMO Panel to comments submitted by the Member States during the three-month consultation period. On 17 January 2019 these two

[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.2019.5521

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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-NL-2013-113;
- The opinion of EFSA;
- The advices already adopted by the BAC on the single events en lower order subcombinations. The conclusions of the BAC for the most recent applications for the single events were as follows:

Event	Application number EFSA/GMO/	BAC advice	Conclusions
MON 89034	NL/2007/37	BAC/2009/880 (03/02/2009)	No major risks for human and animal health or concerning the environment were identified.
1507	RX-001	BAC/2017/0186 (21/03/2017)	Unlikely to pose any risk to human and animal health. No risk identified for the European environment.
MON 88017	CZ/2005/27	BAC/2009/1045 (13/07/2009)	No major risks for human and animal health or concerning the environment were identified.
59122	RX-003	BAC/2017/0740 (19/09/2017)	Unlikely to pose any risk to human and animal health. No risk identified for the European environment.
DAS-40278-9	NL/2010/89	BAC/2017/0066 (31/01/2017)	No conclusion about the food and feed safety of maize DAS-40278-9 in the context of its proposed uses.[in dossier AP151 a 90-day feeding study was added leading to the conclusion that from a toxicology point of view the event is safe]
MON 89034 x MON 88017	NL/2007/39	BAC/2010/0371 (07/05/2010)	No major risks for human and animal health or concerning the environment were identified.
MON 89034 x 1507	BE/2013/118	BAC/2017/0742 (19/09/2017)	Unlikely to have adverse effects on human and animal health and the environment, in the context of its intended uses.
MON 89034 x 1507 x MON 88017 x 59122 (and its 10 subcombinations)	CZ/2008/62	BAC/2010/1159 (17/12/2010)	Unlikely to have adverse effects on human and animal health and the environment, in the context of its intended uses.
1507 x 59122	NL/2005/15	BAC/2009/1366 (02/10/2009)	No major risks for human and animal health or concerning the environment were identified.

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

Scientific evaluation

1. Environmental risk assessment

The Biosafety Advisory Council is of the opinion that it is unlikely that the accidental release of maize MON 89034 x 1507 x MON 88017 x 59122 x DAS-40278-9 (i.e. during transport and/or processing) into the European environment[4] will lead to environmental harm.

2. 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.

[3] See EU register of GM food and feed: http://ec.europa.eu/food/dyna/gm_register/index_en.cfm
[4] As the application doesn't imply cultivation of the GM crop in the EU, a full environmental assessment is as in the case of a cultivation file is not warranted.

3. Assessment of food/feed safety and nutritional value

3.1. 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 five-stacked event, the Biosafety Advisory Council agrees with the GMO panel of EFSA that the compositional data of GM maize MON 89034 x 1507 x MON 88017 x 59122 x DAS-40278-9, in comparison with its conventional counterpart, do not raise safety concerns.

3.2. Assessment of toxicity

The Biosafety Advisory Council has evaluated the safety of the newly expressed Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT, CP4-EPSPS and AAD-1 proteins in the context of previous applications, and no safety concerns were identified. Taking into account the updated information considered in the current application, the Council is of the opinion that its previous conclusions remain valid.
The Biosafety Advisory Council is also of the opinion that the combined expression of the newly expressed proteins in the stacked event does not raise toxicological concerns.

3.3. Assessment of allergenicity

The Biosafety Advisory Council has evaluated the safety of the newly expressed Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT, CP4-EPSPS and AAD-1 proteins in the context of previous applications, and no concerns 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 expression of the newly expressed proteins in the stacked event does not raise concerns regarding the allergenicity.

3.4. Nutritional value

The Biosafety Advisory Council is of the opinion that the information provided is sufficient to conclude that the nutritional characteristics of maize MON 89034 x 1507 x MON 88017 x 59122 x DAS-40278-9-derived food and feed are not expected to differ from those of conventional maize varieties.

4. Monitoring

Since the allergenicity of the whole GM maize has not been fully assessed, it is recommended to take up monitoring of allergenicity as part of the general surveillance.

Conclusion

Based on the whole set of data on maize MON 89034 x 1507 x MON 88017 x 59122 x DAS-40278-9 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 five single events and the lower order subcombinations, the Biosafety Advisory Council:

1) Agrees with the GMO panel of EFSA that the potential environmental release of maize MON 89034 x 1507 x MON 88017 x 59122 x DAS-40278-9, and all its subcombinations independently of their origin, is unlikely to pose any threat to the European environment;
2) Agrees with the GMO panel of EFSA that there is no reason to expect interactions between the newly expressed proteins that could impact on the food or feed safety;

3) Agrees with the GMO panel of EFSA that in the context of its proposed uses, maize MON 89034 x 1507 x MON 88017 x 59122 x DAS-40278-9, and all its subcombinations independently of their origin, is unlikely to pose any risk to human and animal health;
4) Considers that the conclusions of the Biosafety Advisory Council on the single events and lower order subcombinations that have been assessed previously (see table on page 2) remain unchanged.

In addition, the Biosafety Advisory Council recommends following up any unanticipated allergenicity aspects of the GM maize in monitoring systems.

Dr. Corinne Vander Wauven
President of the Belgian Biosafety Advisory Council

Annex I: Compilation of comments of experts in charge of evaluating the application EFSA-GMO-NL-2013-113 and Comments submitted on the EFSAnet on mandate of the Biosafety Council (ref. BAC_2017_0691)

Bioveiligheidsraad
Conseil de Biosécurité

06/09/2017

Secretariaat
Secrétariat

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Compilation of comments of experts in charge of evaluating the application EFSA/GMO/NL/2013/113
and
Comments submitted on the EFSAnet on mandate of the Biosafety Council

Mandate for the Group of Experts: Mandate of the Biosafety Advisory Council (BAC) of 20 June 2017.
Coordinator: René Custers
Experts: Jacques Dommes (ULg), Leo Fiems (ILVO), Johan Grooten (UGent), André Huyghebaert (UGent), Peter Smet (Consultant), Frank Van Breusegem (UGent)
Domains of expertise of experts involved: Molecular characterisation, DNA/RNA/protein analysis, herbicide tolerance, animal and human nutrition, food/feed processing, toxicology, general biochemistry, statistics, immunology, alimentary allergology, plant allergens, agronomy, ecology, oilseed rape, breeding techniques, plant biology.

SBB: Didier Breyer, Fanny Coppens, Katia Pauwels.

- **INTRODUCTION**

Dossier **EFSA/GMO/NL/2013/113** concerns an application submitted by the company **Dow Agrosciences LLC** for authorisation to place on the market genetically modified **maize MON 89034 × 1507 × MON88017 x 59122 × DAS-40278-9 i**n the European Union, according to Regulation (EC) No 1829/2003 on genetically modified food and feed.
The application has been officially acknowledged by EFSA on 21 November 2016 .

The scope of the application is:
☒ 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)

Depending on their expertise, the experts were asked to evaluate the genetically modified plant considered in the application on its 1) molecular, 2) environmental, 3) allergenicity, 4) toxicity and/or 5)

food and feed aspects. It was expected that the expert should evaluate if 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.

The comments are structured as in the "Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed" (EFSA Journal (2004), 99, 1-94). Items are left blank when no comments have been received either because the expert(s) focused on other related aspects, or because for this dossier the panel of experts who accepted to evaluate the dossier didn't have the needed expertise to review this part of the dossier.
It should be noted that all the comments received from the experts are considered in the evaluation of this dossier and in formulating the final advice of the Biosafety Advisory Council. Comments placed on the EFSAnet are indicated in grey.

List of comments/questions received from the experts

GENERAL COMMENTS

Comment 1

MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize may be as safe for human and animal health and the environment as conventional maize, based on the weight of evidence with regard to the toxicological and the allergenic assessment. Results of the compositional analysis showed that some compounds were different between genetically modified and the isoline, but differences were not biologically relevant.
However, based on a holistic approach of MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize the risk assessment should also take the potential for accumulation of residues and metabolites of the herbicides 2,4-D, glyphosate, glufosinate and quizalofop into account, against which MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize is tolerant.

SBB comment :
The evaluation of the safety of the herbicides (or their breakdown products) to which GM maize MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 is tolerant is not within the remit of the Biosafety Advisory Council.
However, In the context of its advice on DAS-68416-4 soybean (**application EFSA/GMO/NL/2011/91, ref WIV-ISP/41/BAC/2017_0437),** the Council verified that the safety of 2,4-D residue and its breakdown product 2,4-Dichlorophenol will be evaluated and a maximum residue level on **soybean** will be set in the framework of Regulation (EC) No 1107/2009.

Comment 2
Maize MON 89034 x 1507 x MON 88017 x 59122 x DAS-40278-9 will be further referred to as maize 113.

Comment 3
No general comment

A. HAZARD IDENTIFICATION AND CHARACTERISATION

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

Comment 1
No remark, adequate information was provided.

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

This GM plant was obtained by traditional breeding from five single events. Southern blot hybridisations were carried out to show convincingly that this stacked GM plant contains intact inserts of MON89034, 1507, MON88017, 59122 and DAS-40278-9 events. I just have a remark concerning the main text (Part II Scientific Information). The plasmid used to generate the 59122 event is referred to as PHP17662 in the text and in fig. 5. However it is named as PHP17661 in the captions of fig. 17 to 27 (Southern blot hybridisations, control genomic DNA spiked with PHP17661). I made the assumption that this was just a typing mistake.

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

No comment, adequate information was provided.

A.3. COMPARATIVE ASSESSMENT

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

Comment 1

Maize 113 is compared with non-GMO control maize with comparable genetic background and six conventional maize hybrids.

No remarks.

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

Comment 1

The experimental design includes unsprayed maize and the application of 2,4-D, glyphosate, glufosinate and quizalofop, against which MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize is tolerant. This is in agreement with the guidelines of EFSA (2010). Consequently, it is evident that this factor should be taken into account for the comparative analysis, including the residue concentrations of 2,4-D, glyphosate, glufosinate and quizalofop in MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize.

It was mentioned that a single outlier was found (P.101 of the Technical Dossier), referring to Appendix C Table 2 of Ekmay (2014). However, Ekmay (2014) and most appendices referred to in the Technical Dossier were not found.

SBB comment :

Ekmay (2014) and most other appendices were made available under Part II scientific information > Appendices- non-CI (scroll to second screen) > Ekmay 2014.

Comment 2

No particular remarks.

A.3.3. COMPOSITIONAL ANALYSIS

Comment 1

Some compounds of MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize were significantly different from conventional maize, but differences are not considered biologically relevant. A variation in the compositional analysis 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 2,4-D, glyphosate, glufosinate and quizalofop in case of MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 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? The risk assessment should take this potential for accumulation into account (FAO, 2009). OECD (2009) mentioned the analysis of toxicants, meaning those toxicologically significant compounds known to be inherently present in the species, whose toxic potency and levels may impact human and animal health.

SBB comment :

See SBB comment under general comments.

Coordinator comment :

The reason to include the treatments with these herbicides is to check whether their application has an effect on the composition of the maize itself (beyond the fact that residues may be present).

Comment 2

Selections of compounds
The OECD guidelines 2002 are followed.
My comments are in line with comments made before in previous applications.
No remarks on:
- proximates, as dietary fiber is included,
- amino acids and fatty acids as relevant compounds are studied.

Vitamins: the whole range of vitamins is assessed; vitamin E is however limited to α-tocopherol and no data are available for the other tocopherols and tocotrienols; it is rather unlikely that the conclusions for α-tocopherol would not apply for the other tocols; for an in depth study of the nutritional equivalence data on the relevant tocols are desirable.
Maize is known to be an good source lutein and zeaxanthin in human nutrition. Information on those compounds is desirable.
relevant

Minerals are studied in detail: relevant minerals are included, even selenium. There are no data on heavy metals such as mercury, cadmium and others.

Secondary metabolites and anti-nutrients: no problem with the compounds studied but information on the potential presence of mycotoxins would be relevant. As mentioned before maize are rather sensitive to the presence of a series of important mycotoxins, relevant for human and animal health.

Field trials on a large scale under different soil and climatologically conditions are an unique opportunity to assess the resistance to disease and to confirm that the inherent sensitivity to these diseases is unaltered or not.

As it is usually the case, analytes are categorized by equivalence/non equivalence and outcome type. The analysis identified 26 analytes as type 3 and higher.

The biological relevance to human health and nutrition was discussed taking into account the European diet. Attention was given to crude protein and amino acids, carbohydrates, stearic acid, linolenic acid, iron, phosphorous, manganese, vitamin A, niacin, magnesium, zinc and ferulic acid.

As an example stearic acid is discussed. The level of stearic acid, a saturated fatty acid, has to be kept as low as possible. A decrease in stearic acid level of maize 113 was observed in comparison to non-GMO maize: 8,3 % on a fatty acid basis. The relevance of this finding was discussed in the context of the European current food intake. I fully agree with the conclusion that this variation has no impact on current saturated fatty acid intake.
I notice that in other applications for GMO maize an opposite trend was observed: an increase rather than a decrease in the stearic acid content. Biological variability is probably at the basis of this observation.

It was concluded that in the context of a complete diet, the observed differences have no biological relevance on humans.

I have no further remarks and I agree with this conclusion.

Coordinator comment :
With regards the remark on the vitamins, we have had discussion on vitamin E before, and I think we concluded that alpha-tocopherol is the most relevant fraction to look at, because it is the substance that provides the biological action.

Coordinator comment :
With regard the remark on the presence of mycotoxins it would be relevant to know when one would want to perform a feeding study. But it is not part of the compositional analysis of the maize itself as it is the result of an external infection.

Comment 3

The amounts of the different proteins under study in the stacked event are comparable to the control lines.

Furfural is missing in both table 35 and 36. What is the category and outcome type of the statistical analysis for this product?

Apart from ferulic acid, there is either no significant difference from the control or equivalence to the reference lines.
For ferulic acid, the differences are small and mean values are higher than the isoline, but lower than the reference line. This seems to be of no major biological relevance.

SBB comment :
Referring to Table 27, the applicant noted that furfural measurements were below the limit of quantification. Therefore no statistical analysis was conducted for this analyte.

A.3.4. AGRONOMIC AND PHENOTYPIC CHARACTERISTICS

Comment 1
The yield of the maize genotypes has only been discussed marginally.

A.3.5. EFFECTS OF PROCESSING

Comment 1
The applicant refers to the processing at high temperatures (up to 195°C; P210 of the Technical Dossier) leading to the denaturation and degradation of the new proteins. Temperatures up to 195°C are high; maximum temperature reported on P. 167-174 of the Technical Dossier was 95°C.

Comment 2
Taking into account that maize 113 is substantially equivalent to conventional maize, no effects on the processing is expected. The applicant states that no novel processes, in wet and dry milling, are envisaged.

I agree with this conclusion.

A.4. TOXICOLOGICAL ASSESSMENT

A.4.1. METHODOLOGY USED FOR TOXICITY TESTS

No comments

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
The chance that the new proteins of MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize (Cry1A.105, Cry2Ab2, Cry1F, CP4 EPSPS, PAT, Cry3Bb1, Cry34Ab1, Cry35Ab1, AAD-1) will pose serious risks for toxicity is negligible, based on the evaluation of these proteins in single events, 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. It is assumed 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 no plausible or testable hypothesis for an interaction of new

proteins in MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 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 toxicity of MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize may also depend on the application of 2,4-D, glyphosate, glufosinate and quizalofop in MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize: see the experimental design involving treatments with the application of 2,4-D, glyphosate, glufosinate and quizalofop. OECD (2009) mentioned the analysis of toxicants, meaning those toxicologically significant compounds known to be inherently present in the species, whose toxic potency and levels may impact human and animal health. Deformed new-born pigs have been reported by Sørensen et al. (2014), and the authors suggested that glyphosate rather than the genetically modified crops were responsible for the deformities.

Agricultural practices using large-scale cultivation of MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize can lead to increasing amounts of sprayed 2,4-D, glyphosate, glufosinate and quizalofop and subsequently of residues in the harvest. Therefore, I think the potential toxicity caused by the residues from spraying with the complementary herbicides is also relevant.

SBB comment :
See SBB comment under general comments.

Coordinator comment :
The toxicity of the IMPORTED PRODUCT may also depend on the application of the herbicides, not the toxicity of the maize itself. The toxicity of possible herbicide residues is within the remit of the pesticides legislation and the MRLs set in that legislation.

Comment 2
No remark. Bioinformatic analyses were repeated on updated databases (2014) and they confirmed the analyses made for the single events.

Comment 3
During earlier studies no acute toxic effect has been demonstrated for cry1A.105, cry2Ab2, cry1F, cry34Ab1, cry35Ab1, cp4 epsps, pat and aad-1.

No acute toxic effects were demonstrated for cry3Bb. Is this the same protein as cry3Bb12?

Earlier studies demonstrated rapid breakdown of these proteins.

Sequence homology with known toxins: All studies were performed in 2013 or 2014. Please provide up-to-date material.

Coordinator comment :
I have screened part II scientific information, and throughout this text it is about Cry3Bb1 (and not cry3Bb or cry3Bb12). I don't know where in the dossier cry3Bb12 is being mentioned.

Coordinator comment :

The dossier was submitted in 2013 (hence its number 2013/113). And then the dossier was processed and questions were asked and answered. I think it is difficult to require sequence homology studies from 2016/2017 for a dossier that was accepted in 2013.

A.4.3. ASSESSMENT OF NEW CONSTITUENTS OTHER THAN PROTEINS

No comments

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

No comments

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

Comment 1
Not performed. No further testing is needed.

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
This is an elaborately documented and well-prepared dossier.

The risk analysis of the individual genetic traits has been updated from the previous applications using FARRP Allergen Database Versions 13 and 14 released in January 2013 and 2014. From this bioinformatics analysis, novel risks for allergenicity of the introduced Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34/35Ab1, PAT, CP4 EPSPS and AAD-1 proteins did not emerge.

While a significant number of newly expressed proteins are stacked in this hybrid maize GMO, I agree with the applicant that the respective molecular action mechanisms make it unlikely that stacking of the individual traits will result in mutual interactions that may increase the risk for allergenicity. Also the comparative compositional analysis performed, points to such a lack of mutual interaction.

Accordingly, I concur with the applicant's conclusion that no concerns in relation to allergenicity of the (stacked) newly expressed proteins were identified.

I have no further comments.

Comment 2
No remark. Bioinformatic analyses were repeated on updated databases (2014) and they confirmed the analyses made for the single events.

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

Comment 1
MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize seems to be comparable with conventional maize with regard to allergenicity. Based on the evaluation of the new proteins in single events it is unlikely that it will pose a serious allergenic risk.

Comment 2
I concur with the applicant's proposition that maize not being considered an allergenic food, the stacking of the individual events is unlikely to alter the overall (lack of) allergenicity of the whole plant. I find it nevertheless a pity and a lost opportunity regarding the establishment of safety that a 90-day feeding study with whole food and feed in rodents has not been performed. Considering the multiplicity of introduced events, such a feeding study would provide in my opinion the best evidence for safety of the hybrid maize plant.

I have no further comments.

A.5.3. ADJUVANTICITY

Comment 1
No comments

A.6. NUTRITIONAL ASSESSMENT

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

No comments

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

Comment 1
There is no reason to assume that the genetic modification affects the nutritional value of the feed derived from MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize. Some compounds of MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize were significantly different from conventional maize. Therefore, a general surveillance should be used to evaluate if the observed differences may affect the nutritional value of the food and feed from MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize in the future.
The applicant mentioned that ruminants necessitate the addition of extra phosphorus to the diet (NRC, 2000; NRC, 2001; P137 of the Technical Dossier), and that the increased phosphorus content of MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize, compared with isoline maize (Table 41), can be considered as beneficial. Dietary phosphorus requirements (g/kg dry matter) are higher in dairy than in beef cattle. Grain-based feedlot cattle diets do not require supplementation of inorganic mineral P to meet P requirements (Erickson et al., 2002; Geisert et al., 2010).

Coordinator comment :

General surveillance is a standard requirement for all GMO products placed on the European market. I see no arguments here to require specific additional monitoring.

B. EXPOSURE ASSESSMENT - ANTICIPATED INTAKE/EXTENT OF USE

Comment 1

An extended combined margin of exposure, as proposed by Wilkinson et al. (2000) and Meek et al. (2011), based on the new proteins (Cry1A.105, Cry2Ab2, Cry1F, CP4 EPSPS, PAT, Cry3Bb1, Cry34Ab1, Cry35Ab1, AAD-1) in MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize and the residues of the herbicides (2,4-D, glyphosate, glufosinate and quizalofop) is lacking.

C. RISK CHARACTERISATION

No comments

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

No comments

E. ENVIRONMENTAL RISK ASSESSMENT

E.1. INTRODUCTION

Comment 1

A side effect of the use of genetically modified MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize may be that it is not sustainable with regard to the pest management. Santos-Amaya et al. (2015) conducted laboratory selections of a Spodoptera frugiperda (Lepidoptera species) strain, which was already resistant to Cry1F maize with pyramided Bt maize expressing Cry1A.105 and Cry2Ab2 proteins. A Spodoptera frugiperda strain was resistant to the pyramided Bt maize after 10 generations of selection. This showed how rapidly resistance to pyramided Bt crops could occur once resistance/cross-resistance to one Bt gene is present. Carrière et al. (2015) mentioned that the concentration of each toxin of a two-toxin pyramid must be high enough to kill at least 95% of susceptible individuals for pyramids to be most effective. Furthermore, two-toxin pyramids are thus expected to be most effective when they kill at least 99.75% of susceptible insects, assuming that each toxin acts independently. In an analysis of nine pest–pyramid combinations, mortality on pyramids met this criterion in only half of the 18 observations These authors stated that in many cases the survival of susceptible insects is greater than the threshold value of 0.25%, and cross-resistance occurs between the toxins in pyramided transgenic Bt crops. Cry1A.105 and Cry2Ab2 proteins have been inserted in MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 maize, the stacked event under investigation.

Coordinator comment :

It is absolutely true that in the field resistant target pests have already emerged which lower the agricultural value of these GM stacked events. This is an important agronomical problem in the countries were this maize will be grown. But in this particular case the dossier is about import of harvested crop into the European Union, and not about cultivation in Europe. Therefore these considerations are not relevant in the context of the current dossier.

Comment 2

No comment

E.2. General approach of the ERA

Comment 1

No comment

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 biosafety concern

E.3.2. Plant to micro-organisms gene transfer

Comment 1

No biosafety concern

E.3.3. Interaction between the GM plant and target organisms

Comment 1

No biosafety concern

E.3.4. Interaction between the GM plant and non-target organisms (NTOs)

Comment 1

No biosafety concern

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

Comment 1

Maize containing pyramided insecticidal proteins Cry1F, Cry1A.105 and Cry2Ab2, as MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize, may be more resistant to a wide range of Lepidopteran larvae compared with single event and non-Bt hybrids (Rule et al., 2014). Notwithstanding the fact that it is not intended to cultivate MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize in the EU, resistance to single event hybrids require attention to the sustainability of the use of herbicide-tolerant genetically modified crops. This is an aspect that deals with the environmental risk assessment. According to Mortensen et al. (2012) and Evans et al. (2015) herbicide mixtures are not a permanent solution to the problem of herbicide resistance, as they do not prevent it on the long run. The selection of MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9 maize may imply the failure of the single events 1507, 59122, MON 88017 and DAS-40278-9 with regard to herbicide tolerance to glufosinate, glyphosate and 2,4-D, respectively, so that a stacked event as MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9 maize becomes necessary.

Glyphosate-tolerant maize adopters use increasingly more herbicides relative to nonadopters, whereas adopters of insect-resistant maize use increasingly less insecticides (Perry et al., 2016). The applicant concluded that risk that importing of MON 89034 × 1507 × MON 88017 × 59122 × DAS-40278-9 in the EU will result in harm to sustainable agricultural production or biodiversity is negligible (P.204 of the Technical Dossier). This is true because cultivation of maize in the EU is not intended. However, Schütte et al. (2017) expected adverse impacts of herbicide-resistant crops on biodiversity when widely adopted, which would be very hard to avoid.

Comment 2

No biosafety concern

E.3.6. EFFECTS ON BIOGEOCHEMICAL PROCESSES

Comment 1

No biosafety concern

E.3.7. EFFECTS ON HUMAN AND ANIMAL HEALTH

Comment 1

Deformed pigs were born out of sows which have been fed glyphosate-resitant soybean meal (Sørensen et al., 2014). 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). Human health concerns with regard to the use of 2,4-D and glyphosate have also been reported (Loomis et al., 2015). Quizalofop-p-ethyl can induce a liver injury (Elefsiniotis et al., 2007).

As residues of herbicides are often found in drinking water (Donald et al., 2007), a good management practice is of utmost importance to restrict these residue concentrations. Even if MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize will not be cultivated in the EU, some alertness is necessary with regard to the use of 2,4-D, glyphosate, glufosinate and quizalofop.

Coordinator comment :

The remark on residues of herbicides in drinking water would be relevant in the context of a cultivation dossier. But the import of GM maize are not likely to contribute to residue levels in drinking water.

Comment 2
No biosafety concern

E.3.8. OVERALL RISK EVALUATION AND CONCLUSIONS

Comment 1
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 MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize on the nutritive value and animal and human health.
Because high doses of 2,4-D and glyphosate may be toxic (Loomis et al., 2015; Williams et al., 2016), the application doses during cultivation should be carefully respected. Even if MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 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 MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize containing herbicide residues may be detrimental for the health of the EU consumer.

SBB comment :
See SBB comment under general comments

Coordinator comment :
Again, this is about pesticide residues and is within the remit of the pesticide legislation and the MRLs set in that legislation.

Comment 2
No biosafety concern

E.4. POST MARKET ENVIRONMENTAL MONITORING PLAN

E.4.1. INTERPLAY BETWEEN ENVIRONMENTAL RISK ASSESSMENT AND MONITORING

No comments

E.4.2. CASE-SPECIFIC GM PLANT MONITORING

No comments

E.4.3. GENERAL SURVEILLANCE FOR UNANTICIPATED ADVERSE EFFECTS

Comment 1
Because of some compounds of MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize were significantly different from conventional cotton (See Section A.3.3), the general surveillance should check whether unexpected adverse effects could occur.

Coordinator comment :
This is a standard requirement for any GMO product placed on the European market.

E.4.4. REPORTING THE RESULTS OF MONITORING

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