Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on application EFSA-GMO-NL-2013-112 (genetically modified maize MON 89034 x 1507 x NK603 x DAS 40278-9) from Dow Agrosciences under Regulation (EC) No. 1829/2003

19 March 2019
Ref. SC/1510/BAC/2019_0248

Context

Application EFSA-GMO-NL-2013-112 was submitted by Dow Agrosciences for the marketing of genetically modified (GM) maize MON 89034 x 1507 x NK603 x DAS 40278-9 (Unique Identifier MON-89Ø34-3 x DAS-Ø15Ø7-1 x MON-ØØ6Ø3-6 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 four-event stack maize MON 89034 x 1507 x NK603 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;
- NK603, expressing the CP4 EPSPS protein and its variant CP4 EPSPS L214P, for tolerance to glyphosate.
- DAS-40278-9, expressing the AAD-1 protein for tolerance to 2,4-D and AOPP herbicides.

The application was validated by EFSA on 29 August 2014. A formal three-month consultation period of the Member States was started on 21 November 2016, lasting until 21 February 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). Ten 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 16 January 2019 (EFSA Journal 2019;17(1):5522[2]), together with the responses from the EFSA GMO Panel to comments submitted by the Member States during the three-month consultation period. On 21 January 2019 these two documents were forwarded to the Belgian experts. They were invited to give comments and to react if needed.

[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.2019.5522

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In delivering the present advice the BAC considered in particular the following information:
- The comments formulated by the experts on application EFSA-GMO-NL-2013-112;
- 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.
NK603	UK/2005/22	BAC/2009/1367 (02/10/2009)	No major risks for human and animal health or concerning the environment were identified.
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 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 NK603	BE/2013/117	BAC/2017/0741 (19/09/2017)	Unlikely to have adverse effects on human and animal health and the environment, in the context of its intended uses.
1507 x NK603	RX-008	BAC/2018/0705 (11/09/2018)	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 NK603	NL/2009/65	BAC/2010/1160 (17/12/2010)	Unlikely to have adverse effects on human and animal health and the environment, in the context of its intended uses.

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 NK603 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. 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 four-stacked event, the Biosafety Advisory Council agrees

[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.

with the GMO panel of EFSA that the compositional data of GM maize MON 89034 x 1507 x NK603 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, PAT, CP4 EPSPS, CP4 EPSPS L214P 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, PAT, CP4 EPSPS, CP4 EPSPS L214P 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 NK603 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 NK603 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 NK603 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 NK603 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-2013-112 and Comments submitted on the EFSAnet on mandate of the Biosafety Council (ref. BAC_2017_0097)

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Conseil de Biosécurité

Secretariaat
Secrétariat

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21/02//2017

Compilation of comments of experts in charge of evaluating the application EFSA/GMO/NL/2013/112 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 December 2016.
Coordinator: Geert Angenon
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), Hadewijch Vanhooren (KUL)
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, breeding techniques, plant biology.
SBB: Didier Breyer, Fanny Coppens, Katia Pauwels.

♦ INTRODUCTION

Dossier **EFSA/GMO/NL/2013/112** concerns an application submitted by the company **Monsanto** for authorisation to place on the market genetically modified **maize MON 89034 × 1507 × NK603 × DAS-40278-9** in 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
No questions

Comment 2
No comments

Comment 3
Genetically modified MON 89034 × 1507 × NK603 × DAS-40278-9 maize may be as safe for human and animal health as conventional maize. Results of the compositional analysis showed some compounds that were different between genetically modified and reference maize varieties, but differences were considered to be not biologically relevant.

It is a pity that the applicant do not use SI units, but lb, oz, qt, pt, A, isref, isGC, …

Comment 4
No comments

Comment 5
Maize 112 or MON 89034 x 1506 x NK 603 x DAS-40278-9 is obtained by crossing maize MON 89034, 1507, NK 603 and DAS-40278-9 using conventional breeding techniques.
It would be quite surprising if any significant difference in composition would be demonstrated as the stacked transformations events have been previously authorized.

Comment 6
References are missing in the folder Part II Scientific Information > Appendices (Non-CI). See details in section A.2.2. In consequence, I could not complete the evaluation of the MC, regarding the following issues: 1) the analysis of the flanking regions and of the ORFs within the insert and spanning the junction sites for the transformation event 1507; 2) the analysis of the flanking regions for the transformation event DAS-40278-9.

The reference Radke (2014d) and Radke (2014e) are missing. These studies report on the up-to-date bioinformatic analysis of the Cry1F and PAT proteins respectively, searching for potential homology with toxins and other bioactive proteins. Hence the evaluation of the bioinformatic characterization of the newly expressed proteins could not be completed.

A. HAZARD IDENTIFICATION AND CHARACTERISATION

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

Comment 1
No questions

Comment 2
Adequate information was provided, no safety concern.

Comment 3
No comments

Comment 4
No remarks

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
No questions

Comment 2
Adequate information was provided, no safety concern.

Comment 3
No comments

Comment 4
No remarks

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
Mon89034x1507xNK603xDAS-40278-9 is produced by traditional breeding between genetically modified Mon8904 expressing Cry1A105 and Cry2Ab2 providing activity against key lepidopteran pests, 1057 maize, providing a third activity and season long protection to lepidopteran pests, and producing PAT providing tolerance to glufosinate-ammonium herbicide, NK603 producing CP4EPSPS providing tolerance to glyphosate, and DAS-40278-9 producing the aryloxyalkanoate dioxygenase enzyme for degradation of the herbicide 2,4 dichlorophenoxyacetic acid (2,4D) into the inactive 2,4 dichlorophenol as well as some others (e.g. quizalofop) into their inactive phenols.
The hybridization patterns for the combined trait product were identical to those of the respective single products as shown by Southern blot analyses and no new genetic modification has been introduced in the combined trait product; no interactions between the inserts.
No further questions; well explained

Comment 2
No comments

Comment 3

No comments.

Page 60: it is stated that '… maize is a segmental allotetraploid…'. From the literature it is known that landraces and cultivars are diploid (2n=2x=20). Also, the CV in the dossier is to my opinion diploid so the relevance of the comments on page 60 should be questioned.

Coordinator's comment :

The maize genome is considered to be the result of an allotetraploidization event 10-15 Mya which since then underwent diploidization.

Comment 4

1.

In page 52 of the technical dossier, the applicant states the inserts are "stably inherited, genotypically and phenotypically" in the stack and refers to different parts of the dossier for substantiating this statement. Regarding phenotypic stability, it is referred to section A.3.4. However, none of the agronomic traits observed in the field and reported in this section correspond to the genetically modified traits. Hence this cannot be regarded as an indication of the phenotypic stability of the transformation events in the stack. However, some evidence is provided in Poorbaugh (2011), Appendices non-CI, and in the section A.2.2.3 describing the expression of the inserts at the protein level. it is surprising that the protein expression studies reported there are not mentioned, which seem a better argument (yet not a proof) for the phenotypic stability of the engineered traits.

2.

References are missing in the folder Part II Scientific Information > Appendices (Non-CI) : Rapier 2014 a and b , Richey 2014b.

The consequence is that I could not check the conclusions regarding: 1) the analysis of the flanking regions and of the ORFs within the insert and spanning the junction sites for the transformation event 1507, 2) the analysis of the flanking regions for the transformation event DAS-40278-9.

Coordinator's comment :

These references indeed seem to be missing from the dossier

3.

Page 54 DAS-40278-9: there is a copy-and-paste mistake in the sentence just before § f), where 1507 is obviously not the transformation event to be mentioned (should be DAS-40278-9).

4.

Protein levels in the stack as compared to the single events: the main dossier presents data on grains only, considering that this is the 'primary source' of the maize stack that will be used in the EU. If my memory is correct, in previous dossiers of imported maize, data on forage are also analysed, hence data on forage were expected here as well. However, these data can be found in Maldonado (2012), raising no concerns, which should have been inserted in the protein expression tables of the main dossier.

A.3. COMPARATIVE ASSESSMENT

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

Comment 1
No questions

Comment 2
As in previous similar applications 112 maize is compared to a non-GMO near isogenic comparator. Six commercial maize hybrids were also included in the study.

Comment 3
No remarks

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

Comment 1
The product was compared with non-GM control maize (near isogenic), and was compared with 6 conventional maize hybrids as reference substances.
Reference hybrids were randomized across sites in an incomplete block design with 3 reference lines at each site, and each reference line present at 5 sites in all cases (p74); in the text it is mentioned 4 to 6 sites as shown in table 17, but according to table 17 it seems to me at 5 sites for each reference line

No further questions

Comment 2
No particular remark on the selection of the comparators, the study design, the field site, the application of fertilizers and pesticides and the sampling.
The statistical analysis was performed according to the EFSA guidelines.

Comment 3
No remarks

A.3.3. COMPOSITIONAL ANALYSIS

Comment 1
-Table 32: why Se (selenium) is not in the table for selection of compounds for analysis? See table 34 where Se is characterized under category I and see also discussion on p82 about Se-analysis.

-Table 38: values are expressed as mg/kg but for manganese it is as mg/100g?? Is this correct? This would implicate that the content of Mn is 10 times higher as Iron which seems unlikely.
It is also not consistent with values of minerals given in table 42, p132; here iron is much higher than manganese and amounts in table 42 are given in mg/kgDW. For manganese the values in table 42 (mg/kg DW) correspond with those in table 38 (in mg/100g), but then values for iron are not correct in table 38 (100 times factor different). Check this please!!

-p112: under Vit B6, a minor decrease in VitB6 content of GM maize was observed in comparison to isoline maize. However this decrease accounts for only a 0.02 mg/d decrease in manganese intake?? Should it be decrease in VitB6 intake instead??

Coordinator's comment :
Typographical error: should be VitB6

-p122 what about the use of phytase? Does it affect Fe-needs??

Comment 2
No questions

Comment 3
Twenty-one analytes were categorised as type 3 or higher according to the statistical assessment of differences and equivalences between genetically modified and reference maize varieties (van der Voet et al., 2011). This is somewhat amazing because the history of the agricultural fields was taken into account; maybe a soil scan could have improved the estimation of the homogeneity of the parcels. However, differences between genetically modified and reference plant varieties are not biologically relevant.

It is regrettable that residues of 2,4-D, glyphosate and glufosinate herbicides and metabolites were not reported, or referred to. The controversy with regard to the safety of glyphosate (EFSA, 2015; Guyton et al., 2015) can be considered as an element of the risk assessment

SBB comment:
The assessment of the safety of glyphosate and glufisonate herbicides and metabolites are not within the remits of the BAC.

Comment 4
Compounds in grain selected for analysis, according to the OECD guidelines:
proximates and fiber:
minerals: no remarks
amino acids: essential amino acids are included
fatty acids: essential fatty acids, particularly the relevant polyunsaturated fatty acids are included
vitamins: the assessment covers the relevant vitamins;
- maize is a good source of tocopherols; only α-tocopherol is included, but not the other tocopherols and tocotrienols; maize is a good source of tocotrienols with good antioxidative properties;
- provitamin A is included in the list however maize contains some important other carotenoids such as zeaxanthin and others.
secondary metabolites: no remarks.

General remark: the OECD list is appropriate for comparative purposes however an update is necessary taking into account actual insights in human nutrition.

Results of the analysis are summarised and characterized by equivalence or non equivalence.
Most analytes are classified in category I or equivalence likely, in category II or equivalence more likely than not.

Analytes for which equivalence with the reference varieties was not demonstrated are discussed in terms of biological significance. Some analytes showed a significant between 112 maize and the isoline and were categorized as different from the isoline. Some of these analytes, such as phenylalanine and other essential amino acids, iron, β-carotene, folic acid, and linoleic acid are really important in human nutrition.

The applicant discusses these observations in terms of biological relevance to human health and nutrition.
The discussion starts with data of the maize consumption in the EU. Particular attention is given to the relevance of some nutrients: crude protein, amino acids, carbohydrates, linoleic acid, iron, manganese, pro vitamin A, vitamin B6, vitamin B9 and others.
The applicant concludes that the observed changes are not biologically relevant in the context of normal EU intake.

I have no objections to these conclusion

Comment 5
The amount of protein in MON 89034 x 1507 x NK603 x DAS-400278-9 is comparable to that in MON 89034 x 1507 x NK603 and DAS-400278-9.

Concerning the presence of antinutrients and secondary metabolites, no biological relevant differences were observed.

Comment 6
No remarks

A.3.4. AGRONOMIC AND PHENOTYPIC CHARACTERISTICS

Comment 1
The applicant discusses the differences in agronomic characteristics and the biological significance.

As far as I could conclude from the documents, there are no data available about the presence of particular mycotoxins.
Mycotoxins in food and feed, among others in maize as a consequence of plant diseases, are a point of attention in human and animal nutrition.

SBB comment:
The assessment of the presence of mycotoxins in particular is not comprised within the scope of the assessment of biologically relevant change(s) as regards compositional, potential toxicity, allergenicity, agronomic and phenotypic characteristics of the GM plant and/or derived food and feed resulting from the genetic modification.

Comment 2
No remarks

A.3.5. EFFECTS OF PROCESSING

Comment 1

I agree with the conclusion that none of the statistically significant differences in analytes were meaningful from a food and feed safety or nutritional perspective.

Comment 2
Maize 112 is substantially equivalent to conventional maize. Particular effects on wet and dry milling are not to be expected.

A.4. TOXICOLOGICAL ASSESSMENT

A.4.1. METHODOLOGY USED FOR TOXICITY TESTS

Comment 1
No questions

Comment 2
No new toxicity (animal) tests were performed in the context of the application of the stacked maize MON89034 x1507 x NK603 x DAS-40278-9 produced by conventional breeding.

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
Mode of action is extensively and well explained; no further questions.

Comment 2
Adequate information was provided, no safety concern.

Comment 3
The chance that the new protein of MON 89034 × 1507 × NK603 × DAS-40278-9 maize will pose serious risks for toxicity is negligible. It can be 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 MON 89034 × 1507 × NK603 × DAS-40278-9 maize (Steiner et al., 2013).

Comment 4
No comments

Comment 5
No new toxicity (animal) tests were performed in the context of the application of maize MON89034 x1507 x NK603 x DAS-40278-9. However all expressed proteins were discussed for the respective applications for the authorisation of the 4 individual GM maize: MON89034, 1507, NK603, DAS-40278-9. The 6 proteins were assessed by EFSA and positive scientific opinions were adopted for MON89034, 1507, NK603, MON89034 x 1507 x NK603, and also recently for DAS-40278-9.

Updated bioinformatics analyses were performed for all 6 expressed proteins: Cry1A.105, Cry2Ab2, Cry1F, Pat, CP4 EPSPS, AAD-1. No homology with known toxins were detected (data from document Part II – scientific Information - of the application). The original Updated Bioinformatics Evaluation reports for the Cry1F (Radke, 2014d), PAT (Radke, 2014e) and AAD-1 (Radke, 2014a) proteins was not made available, and consequently these original reports could not be assessed.

Coordinator's comment :
Radke, 2014d and Radke 2014e are missing from the dossier, Radke 2014a is available

Potential interactions between the Cry1A.105, Cry2Ab2, Cry1F, Pat, CP4 EPSPS, AAD-1 proteins: not expected, no evidence of mechanism of interaction. The potential for interaction among the Cry1A.105, Cry2Ab2, Cry1F proteins was already assessed in previous dossiers.

Comment 6
A bioinformatic search for homology is conducted but is not up-to-date. Studies were performed in 2013 and 2014.
No other tests are needed at this moment.

Comment 7
1.
The reference Radke, 2014d is missing. This study reports on the up-to-date bioinformatic analysis of the Cry1F protein, searching for potential homology with toxins and other bioactive proteins.

2.
The reference Radke, 2014e is missing. This study reports on the up-to-date bioinformatic analysis of the PAT protein, searching for potential homology with toxins and other bioactive proteins.

A.4.3. ASSESSMENT OF NEW CONSTITUENTS OTHER THAN PROTOEINS

Comment 1
Not applicable

Comment 2
No comments

Comment 3
No comments or questions.

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

Comment 1
No questions

Comment 2
The compositional analysis was discussed in document Part II of the application. On the basis of the data in document part II the observed differences in composition can be regarded as not biologically relevant. However, the compositional analysis study (Ekmay, 2014) was not made available, and consequently could not be assessed.

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

Comment 1

No questions

Comment 2

No whole feed studies were performed.

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

Adequate bioinformatics analyses were carried out. No safety concern.

Comment 3

The stacked events lead to the combined expression of Cry1A.105, Cry2Ab2, Cry1F, PAT, CP4 EPSPS and AAD-1 proteins. All these proteins have been assessed individually before in the context of previous applications. No indications pointing towards an increased risk for allergenicity were then identified by EFSA. As these dossiers date back to 2005-2007, the applicants updated the amino acid sequence homology comparison between the newly expressed proteins and known allergens using 2013-2014 databases. The results of this updated analysis indicate that no biologically relevant sequence similarities are present between the Cry1A.105, Cry2Ab2, Cry1F, PAT, CP4 EPSPS and AAD-1 proteins and allergens listed in these databases.

Question: *The single event GM maize strains being already on the market for quite some time, shouldn't the applicants then present data from the monitoring plan and consumer health reports? This would allow to include in addition practice-based opinions.*

There are no indications that the sequences would be intrinsically unstable when stacked together by traditional breeding and/or engage in unintended interactions, hereby affecting the expression levels of the proteins.

Conclusion: *I agree with the applicant's conclusion that no concerns in relation to allergenicity of the (combined) newly expressed proteins were identified. However, I regret the lack of inclusion of data from health reports and monitoring.*

Comment 4

No remarks

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

Comment 1
No questions

Comment 2
The chance that the new protein of MON 89034 × 1507 × NK603 × DAS-40278-9 maize will pose serious risks for allergenicity is negligible.

Comment 3
No remarks

A.5.3. ADJUVANTICITY

Comment 1
No questions

Comment 2
No remarks

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
There is no reason to assume that the genetic modification affects the nutritional value of the feed derived from MON 89034 × 1507 × NK603 × DAS-40278-9 maize.

B. EXPOSURE ASSESSMENT - ANTICIPATED INTAKE/EXTENT OF USE

Comment 1
No questions.

C. RISK CHARACTERISATION

Comment 1
No questions.

Comment 2
Page 182 of the main dossier “Description and quantification of uncertainties” : this task and the description of the assumptions made in characterizing the risks should be part of this section on ‘Risk

Characterization', as laid down in the implementing regulation No 503/2013. However, in this dossier, the applicant repeats the conclusions of the risk assessment and provides no insight on the uncertainties and assumptions made. There is an issue that EFSA has, to the best of my knowledge, not provided guidelines on how to address these uncertainties in the RA, but the situation as it stands now seems not satisfactory.

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

Comment 1
No questions.

Comment 2
No remarks

E. ENVIRONMENTAL RISK ASSESSMENT

E.1. INTRODUCTION

Comment 1
No questions.

Comment 2
No remarks

E.2. GENERAL APPROACH OF THE ERA

Comment 1
No questions.

Comment 2
No comment.

Comment 3
No remarks

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
Ok, no safety concern.

Comment 3
No remarks

E.3.2. PLANT TO MICRO-ORGANISMS GENE TRANSFER

Comment 1
No comment.

Comment 2
Ok, no safety concern.

Comment 3
No remarks

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

Comment 1
No questions.

Comment 2
Ok, no safety concern.

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

Comment 1
No questions.

Comment 2
Ok, no safety concern.

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

Comment 1
Not applicable.

Comment 2

Ok, no safety concern.

Comment 3
Maize containing pyramided insecticidal proteins Cry1F, Cry1A.105 and Cry2Ab2, as MON 89034 × 1507 × NK603 × 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 cultivated MON 89034 × 1507 × NK603 × 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.

SBB comment:
The assessment of the safety of glyphosate and glufisonate herbicides and metabolites are not within the remits of the BAC.

E.3.6. Effects on biogeochemical processes

Comment 1
Not applicable.

Comment 2
Ok, no safety concern.

E.3.7. Effects on human and animal health

Comment 1
Safety of these proteins expressed in the stacked event is established on their:
-lack of acute toxicity as determined in mouse gavage studies
-rapid digestion in simulated gastric fluids
-lack of homology with known protein toxins
-lack of homology with known allergens

Comment 2
Ok, no safety concern.
Comment 3 **(Fiems)**
No detrimental effects of MON 89034 × 1507 × NK603 × DAS-40278-9 maize on human and animal health are expected.

Comment 3
No remarks

E.3.8. Overall risk evaluation and conclusions

Comment 1
No questions.

Comment 2
Ok, no safety concern.

E.4. Post Market Environmental Monitoring plan

E.4.1. Interplay between environmental risk assessment and monitoring

Comment 1
No questions.

Comment 2
No remarks

E.4.2. Case-specific GM plant monitoring

Comment 1
No comments.

Comment 2
No remarks

E.4.3. General surveillance for unanticipated adverse effects

Comment 1
No comments.

Comment 2
No remarks

E.4.4. Reporting the results of monitoring

Comment 1
No comments.

Comment 2
No remarks

References

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