Bioveiligheidsraad
Conseil de Biosécurité

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Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2017_0741

Advice of the Belgian Biosafety Advisory Council on application EFSA-GMO-BE-2013-117 from Monsanto Europe S.A./N.V. under Regulation (EC) No. 1829/2003

Context

Application EFSA-GMO-BE-2013-117 was submitted by Monsanto Europe S.A./N.V. on 13 September 2013 for the marketing of genetically modified (GM) maize MON87427 x MON89034 x NK603 and its subcombinations for food and feed uses, import and processing (excluding cultivation) within the European Union (EU), within the framework of Regulation (EC) No. 1829/2003[1].

The three-event stack maize MON87427 x MON89034 x NK603 was obtained by conventional crossing (no new genetic modification involved) of the corresponding single events:
- MON87427, expressing CP4 EPSPS for glyphosate tolerance;
- MON89034, expressing Cry1A.105 and Cry2Ab2 protein for resistance to lepidopteran pests;
- NK603, expressing the CP4 EPSPS protein and the variant CP4 EPSPS L214P for glyphosate tolerance.

The application was officially acknowledged by EFSA on 22 January 2014. On 11 June 2015 EFSA started the formal three-month consultation period of the Member States, 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 Biosafety and Biotechnology Unit (SBB). Eight 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.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 28 June 2017 (EFSA Journal 2017;15(8):4922[2]), and published on 1st August 2017 together with the responses from the EFSA GMO Panel to comments submitted by the Member States during the three-month consultation period.

[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/4922

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On 4 September 2017 the opinion of EFSA was forwarded to the Belgian experts. They were invited to give comments and to react if needed.

In delivering the present advice the Biosafety Advisory Council considered in particular the information below:
- The comments formulated by the experts on application EFSA-GMO-BE-2013-117;
- The opinion of EFSA;
- The advices already adopted by the BAC on the single events and one stacked event. The conclusions of the BAC were as follows:

Event	Application number	BAC advice	Conclusions
MON87427	EFSA-GMO-BE-2012-110	BAC/2015/0585 (8-09-2015)	No major risks for human and animal health or concerning the environment were identified.
MON89034	EFSA-GMO-NL-2007-37	BAC/2009/880 (03-02-2009)	No major risks for human and animal health or concerning the environment were identified.
NK603	EFSA-GMO-NL-2005-22	BAC/2009/1367 (02-10-2009)	No major risks for human and animal health or concerning the environment were identified.
MON89034 x NK603	EFSA-GMO-NL-2007-38	BAC/2009/1492 (06-11-2009)	No major risks for human and animal health or concerning the environment were identified.

All GM maize 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 MON87427 x MON89034 x NK603 seeds (i.e. during transport and/or processing) into the European environment[4] will lead to any unwanted effects.

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, for the subcombinations previously assessed as well as for the subcombinations that were not previously assessed.

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 three-stacked event, the Biosafety Advisory Council agrees with the GMO panel of EFSA that the compositional data of GM maize MON87427 x MON89034 x NK603, 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 CP4 EPSPS, Cry1A.105 and Cry2Ab2 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.

[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 a cultivation of the GM crop in the EU, a full environmental assessment is not required in EFSA procedure and was not achieved.

The Biosafety Advisory Council is also of the opinion that the combined expression of the newly expressed proteins in the stacked event should not raise toxicological concerns.

3.3. Assessment of allergenicity

The Biosafety Advisory Council has evaluated the safety of the newly expressed CP4 EPSPS, Cry1A.105 and Cry2Ab2 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 87427 x MON 89034 x NK603-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 scientific assessment of the dossier done by the Belgian experts, taking into account the opinion of EFSA, the advices already adopted by the BAC on the three single events and one of the possible subcombinations, and considering the data presently available, the Biosafety Advisory Council:

1) Agrees with the GMO panel of EFSA that the potential environmental release of maize MON87427 x MON89034 x NK603 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 MON87427 x MON89034 x NK603 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 that have been assessed previously (MON87427, MON89034 and NK603, 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 the existing allergenicity monitoring systems.

Prof. Maurice De Proft
President of the Belgian Biosafety Advisory Council

Annex I: Compilation of comments of experts in charge of evaluating the application EFSA/GMO/BE/2013/117 and Comments submitted on the EFSAnet on mandate of the Biosafety Council (ref. BAC_2015_0590)

Bioveiligheidsraad
Conseil de Biosécurité

09/09/2015

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC_2015_0590
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Compilation of comments of experts in charge of evaluating the application EFSA/GMO/BE/2013/117
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 22 June 2015.
Coordinator: Dr. Michel Van Koninckxloo
Experts: Eddy Decuypere (KUL), Leo Fiems (ILVO), Johan Grooten (UGent), André Huyghebaert (UGent), Peter Smet (Consultant), Frank Van Breusegem (UGent), Jan Van Doorsselaere (VIVES), Michel Van Koninckxloo (HEP Hainaut-Condorcet).
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.
SBB: Didier Breyer, Fanny Coppens, Martine Goossens, Katia Pauwels.

♦ INTRODUCTION

Dossier **EFSA/GMO/BE/2013/117** concerns an application submitted by the company **Monsanto** for authorisation to place on the market genetically modified **MON 87427 × MON 89034 × NK603 maize** 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 11 June 2015.

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
Single events dealing with CP4 EPSPS and Cry1A.105 and Cry2Ab2 proteins have already been assessed and EFSA concluded that they are safe for human and animal health. It is assumed that there is no plausible or testable hypothesis for an interaction of the newly-inserted proteins. Consequently, the genetic modification of MON 87427 × MON 89034 × NK603 maize is no reason to prohibit its import and processing in the EU.

A side effect of MON 87427 × MON 89034 × NK603 maize may be an increased use of the herbicide glyphosate, and some health concerns about glyphosate have been reported. MON 87427 × MON 89034 × NK603 is not intended for cultivation in the EU. Nevertheless, introduction of the MON 87427 × MON 89034 × NK603 maize elsewhere may increase the use of glyphosate.

SBB Comment:
The assessment of the use and safety of pesticides is not within the remits of the Biosafety Advisory Council.

Comment 2
The inherited DNA sequences (by conventional breeding) in MON 87427 × MON 89034 × NK603 are coding for agronomic traits which do not alter the phenotypic characteristics nor the compositional analysis of maize.
The CP4EPSPS, Cry1a-105, Cry2ab2 expression cassettes encode the CP4EPSPS, Cry 1A105 and Cry2Ab2 proteins providing tolerance to glyphosate herbicides as well as insect protection against European corn borer and some lepidopteran insect pests. It is clearly stated and argumented that there is no need for further analysis to show the lack of toxicity of the CP4EPSPS, Cry1A105 and Cry2Ab2 proteins, and a new 90-day feeding study in rodents is not needed.

Comment 3
The information provided in the application is sufficient.

Comment 4
No comment.

A. HAZARD IDENTIFICATION AND CHARACTERISATION

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

Comment 1
No questions.

Comment 2
The information provided in the application is sufficient.

Comment 3
No comment.

Comment 4
No comment.

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
Not applicable because MON 87427 × MON 89034 × NK603 is a stacked event and produced through conventional breeding. It expresses the CP4EPSPS proteins from MON 87427 and from MON 89034 × NK603 and the Cry1A.105 and Cry2Ab2 proteins from MON 89034 × NK603. These are assumed as being safe and without concerns for humans, farm animals and environment.
There is also no evidence of any safety issues related to the use of MON 87427 and MON 89034 × NK603 for any of the donor organisms of the coding and non-coding DNA sequences present in MON 87427 and MON 89034 × NK603.
The DNA fragments presented in MON 87427 × MON 89034 × NK603 are inherited from both as stated above and therefor also present no safety issues.

Comment 2
The information provided in the application is sufficient.

Comment 3
The dossier describes the stacking of different genes in maize using conventional breeding. Event MON87427 has been approved for authorisation (EFSA-GMO-BE-2012-110). Event MON 89034 x NK603 has been approved for authorisation (EFSA-GMO-NL-2007-38). It is shown that the structure of the independent T-DNA's is conserved and that gene expression of the different transgenes is similar in the stacked maize as compared to the independent events.

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
The combination of MON 87427and MON 89034 × NK603 by conventional breeding and resulting in MON 87427 × MON 89034 × NK603 results in a plant with an additional insect-resistance by the combination of 2 Cry-proteins (Cry1A.105 and Cry2Ab2) and with full vegetative and male reproductive tolerance to glyphosate in MON 87427 and MON 89034 × NK603 because of full vegetative and female expression of CP4 EPSPS in MON 87427 combined with male reproductive expression of CP4 EPSPS in NK603.

Comment 2
General comment on the bio-informatic analysis p 41 - 52
Although the methodology for analyzing the flanking sequences is solid (searching databases using different search tools) and the separate events (eg MON 87427 and MON 89034 x NK603) have been approved by EFSA, I have the following remark:

The maize genome sequence is available in Genbank and has been annotated (also at ZmGDB): the dossier could benefit from using this information and providing data on the specific location of the T-DNA inserts on the chromosomes and using the annotation data provided in ZmGDB. The dossier could benefit from a figure showing annotation of the flanking sequences e.g. the presence of ORFs (fi >100 bp) or other elements (and the direction of these elements, if relevant). This could be more comprehensive then providing numerous pages, documents, lists, … with the description of database searches.
One should also keep in mind that these *in silico* analyses do not allow to draw unambiguous conclusions. Also if for instance a T-DNA is inserted in a gene, it is possible that it has no effect on the plant (phenotype, protein production, …). For instance in event MON 88017 the T-DNA is inserted in GI-413923506 and this has no effect on phenotype, agronomical and compositional properties. This event has been approved.

A general remark to SBB: should it be asked to the applicant to provide data on the proteome. Several techniques exist such as 2D-CE/GE (in combination with MS) which allow to obtain insights in the presence of proteins and such techniques could be used to compare transgenic with non-transgenic comparators. Such analyses could be more informative than in silico bio-informatic analyses.

The analysis of the genomic DNA – T-DNA insert sequences for putative translated polypeptides which would share homology with for instance allergens, is relevant.

SBB Comment:
The feasability of OMICS technologies as a tool supporting GMO risk assessment is currently under investigation (e.g. EU research project GRACE).

Comment 3
No comment.

A.3. COMPARATIVE ASSESSMENT

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

Comment 1
Ok, no questions.

Comment 2
MON 87427 x MON 89034 x NK603 is obtained by conventional breeding of three varieties, which have been found to be compositional equivalent to conventional maize. Substantial differences in composition are not to be expected.

MON 87427 x MON 89034 x NK603 is compared to a conventional maize variety with a similar genetic background. Other conventional reference maize hybrids are also included in the study.

No particular remarks.

Comment 3
The information provided in the application is sufficient.

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

Comment 1
Ok.

Comment 2
The information provided in the application is sufficient.

A.3.3. COMPOSITIONAL ANALYSIS

Comment 1
A total of 60 of the 65 components assessed were available for statistical analysis, and were not different from the set of commercial conventional reference hybrids at the 95% confidence level. The other component differences were not relevant from a food and feed perspective because the values of MON 87427 × MON 89034 × NK603 maize and the control extensively overlapped. Therefore, differences are not really relevant from a food and/or feed safety perspective.

Comment 2
Nutrient levels in grain: when MON 87427 × MON 89034 × NK603 treated with glyphosate are compared with the near isogenic parental line, 2 amino acids, arginine and glycine were identified as significantly different from control; but these are just 2 non-aromatic amino acids while the resistance to glyphosate just concerns the shikimic acid pathway for the biosynthesis of aromatic amino acids. Moreover in the comparison of MON 87427 × MON 89034 × NK603 non-treated with glyphosate compared with control, only arginine levels in grain were significantly different at the 10°C level. Since all found differences are within the range of variability of individual replicate values for control and certainly within the ranges generated of commercial varieties published in scientific literature, the conclusions about compositional equivalence of MON 87427 × MON 89034 × NK603 to the control in levels of these components is warranted and some small differences found with the near isogenic parental control line are of no relevance from a load and feed perspective.
- This holds also for fat and fatty acids, vitamines, proximates (ash, moisture), carbohydrates, fibers and minerals.
- P.73, second last paragraph: “The inserted traits in MON 87427 × MON 89034 × NK603 are not a major contributor to variability in vitamin levels in maize forage...” Probably this is a typing error and must be in maize grain (or grain and forage); it is not so important in the context, but I think it is better corrected here if I am right.
- The reasoning as for the constituents mentioned above, also holds for anti-nutrient levels in grain or secondary metabolic levels in grain or nutrient levels in forage.

Comment 3
The overall approach is similar to the approach in previous applications.
As it is usually the case, the OECD guidelines are used as a basis for the selection of key nutrients and other relevant components.

Compositional analyses were conducted for:
- proximates acid detergent fibre, neutral detergent fiber, total dietary fiber, ash, carbohydrates by difference
- aminoacids, fatty acids,
- vitamins: β-carotene, B1, B2, B6, E, niacin and folic acid,
- minerals: calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc,
- anti-nutrients: phytic acid, raffinose,
- secondary metabolites: furfural, ferulic acid, and p-coumaric acid.
Relevant components are included.

In previous application the “feed” approach was always selected for the assessment of fiber: acid detergent en neutral detergent fiber. To my knowledge this is one of the first applications where dietary fiber, important in human nutrition, is also included.

On the other hand I prefer a direct analysis of carbohydrates instead of the obsolete approach "carbohydrates by difference".

Results are discussed in detail for:
- MON 87427 x MON 89034 x NK603 treated with glyphosate,
- MON 87427 x MON 89034 x NK603 non treated with glyphosate.

In the statistical evaluation most components were found to be equivalent or equivalent more likely than not. A reasonable explanation is given for components categorized as non-equivalent.

I agree with the overall conclusion that the introduced traits are not major contributors to compositional variability in maize.

Comment 4
The amount of CP4 EPSPS protein in this hybrid is higher than in MON 87427 and NK603 due to the presence of two copies of the *cp4 epsps* gene. This seems to be of no concern since the calculated MOE exceeds a factor 1000.
Both other proteins are present in grain in the same amount as in the parent line with a single event. Furthermore, there seem to be no problem concerning the amounts of anti-nutrients and secondary metabolites in grain.

Comment 5
The information provided in the application is sufficient.

A.3.4. AGRONOMIC AND PHENOTYPIC CHARACTERISTICS

Comment 1
P.90; Athropod Damage: it is stated that in the individual-site assessment, no differences were observed between MON 87427 × MON 89034 × NK603 and the conventional control for any of the 45 compositions for the assessed arthropods. But what about lepidopteran damage? Maize borer damage? This could be of relevance to look for in the program in order to demonstrate the effectiveness of the Cry-gene insertions.

Comment 2
In section 3.4.3 Environmental Interaction evaluations it is mentioned that no differences were observed between MON 87427 X MON 89034 X NK603 and the conventional control for any of the 102 comparisons for the assessed diseases.

This is an important observation as maize is quite sensitive to the formation of mycotoxins, due to the presence of particular moulds. According to these observations there is no increased risk for mycotoxins due to the introduction of the particular traits in maize.

No further remarks.

Comment 3
The information provided in the application is sufficient.

A.3.5. EFFECTS OF PROCESSING

Comment 1
No questions or comments.

Comment 2
The applicant states that, with the exception of the introduced traits, MON 87427 X MON 89034 X NK603 is not different from conventional maize. Any difference in the processing of maize is not to be expected.

I agree with this statement.

Comment 3
The information provided in the application is sufficient.

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
Based on the weight of evidence in this dossier it is unlikely that MON 87427 × MON 89034 × NK603 maize will pose serious risks for toxicity. Single events MON 87427 (EFSA, 2015) and MON 89034 ×

NK603 maize (EFSA, 2009) are as safe as their conventional counterparts and non-GM reference varieties with respect to potential effects on human and animal health and the environment. However, the conclusion from the applicant that Cry1A.105, Cry2Ab2 and CP4 EPSPS proteins have no synergistic or antagonistic effects to each other, or that there is no interaction (see Technical dossier: P.99, P.105, P.106, P.107) is not clearly demonstrated. There is some controversy with regard to the effect stacked events. Kok et al. (2014) stated that by default no further assessment is required for plants with stacked GM events for food and feed safety assessment. Zdziarski et al. (2014) reported an incomplete picture regarding the toxicity and safety of stacked GM crops consumed by humans and animals, while Agapito-Tenfen et al. (2014) stated that there is a lack of data of a kind that might be important in order to reliably assess the safety of stacked GM events. Because there is no biological pathway in which the newly-inserted genes would directly or indirectly interact, there is no plausible or testable hypothesis for the interaction of these proteins in MON 87427 × MON 89034 × NK603 maize (Steiner et al., 2013).

Comment 2
I agree with the conclusions about toxicity studies using laboratory animals. No need for these since
- the history of safe use for the newly expressed proteins
- lack of structural or functional relationship to proteins that adversely affect human or animal health
- low expression levels in grain
- readily digestible in simulated gastric and intestinal fluids
- lack of acute toxicity of these proteins at doses several orders of magnitude higher than anticipated human or farm exposure.

Comment 3
Earlier studies have demonstrated that CP4 EPSPS, Cry1A.105 and Cry2Ab2 are readily digestible in SGF and SIF.
Several acute oral toxicity studies with CP4 EPSPS, Cry1A.105 and Cry2Ab2 proteins have demonstrated that these proteins are not acutely toxic and do not cause any adverse effects.
The results of the bioinformatic analyses demonstrated that no structurally relevant similarity exists between the CP4 EPSPS, CP4 EPSPS L214P, Cry1A.105 and Cry2Ab2 proteins and any known toxic, allergen or other biologically active proteins that would be harmful to human or animal health.
28 Day Repeat Dose Toxicity Study by Oral Gavage in Rats: Not performed. No further testing is needed.

Comment 4
No comment.

A.4.3. ASSESSMENT OF NEW CONSTITUENTS OTHER THAN PROTEINS

Comment 1
Not applicable.

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

Comment 1
No questions

Comment 2
The information provided in the application is sufficient.

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

Comment 1
No questions

Comment 2
90-Day rat feeding study: Not performed. No further testing is needed.

Comment 3
The information provided in the application is sufficient.

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
Based on the weight of evidence in this dossier it is assumed that MON 87427 × MON 89034 × NK603 maize does not pose a serious allergenic risk, and that it is comparable with conventional maize with regard to allergenicity. However, appropriate labelling is desirable for food and feed derived from MON 87427 × MON 89034 × NK603 maize: the consumers have the right for information (Cheftel, 2005). This is not in agreement with the vision of the applicant: see Technical dossier: P.112.

Comment 2
No questions

Comment 3
This hybrid GMO maize combines Cry1A.105 and Cry2Ab2 proteins, providing resistance to certain insect pests, with two CP4 EPSPS proteins, providing tolerance to glyphosate herbicides. Each of the individual traits have been evaluated before by EFSA in independent dossiers for their risk of allergenicity. These individual assessments have been performed according to the requirements from EFSA and did not indicate an increased risk for allergenicity. Some of these dossiers being quite old, the applicant updated the bioinformatics analyses of the introduced proteins using 2013 databases. Also these updated analyses did not point to an increased risk of allergenicity when looking at the single events.
When looking at potential risks resulting from the combined events, two genes encoding CP4 EPSPS proteins cannot be considered as combining two independent traits. Although allegedly both CP4 EPSPS genes do not mutually interfere, their combined action is clearly reflected in the CP4 EPSPS protein expression levels which in grain are double compared to either single trait. This increment may be considered as biologically relevant with an increased risk for allergenicity of the stacked event. Given that this hybrid GMO expresses CP4 EPSPS protein also in pollen, the remarks made in dossier BAC2009_01367 relating to inhalant allergy to maize pollen remain a concern. Also, pollinating honeybees will convert the pollen in honey which serves directly and without (denaturing) heat processing procedures as human food. Also this feature raises a safety concern that is not

addressed by the applicants (*). With the exception of these two remarks, I agree with the applicant's overall conclusion that the introduced proteins are unlikely to increase the risk of allergenicity.

SBB Comment:
(*) The same comment was indeed sent to EFSA in 2006 in the frame of the evaluation of application EFSA/GMO/NL/2005/22.
In summary, EFSA answered that this issue did not appear to be a safety concern to the GMO Panel since maize is not considered a major allergenic food.
In the same line the Biosafety Council did not identify any safety issue with regards to allergenicity in its final advice (BAC_2009_01367). There is only a request for general surveillance (as for all GM maize dossiers).

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

Comment 1
No questions

Comment 2
Maize being a food that only marginally elicits allergies, it is unlikely that the newly introduced traits will alter the overall lack of allergenicity of the plant (provided the introduced traits are non-allergenic).

A.5.3. ADJUVANTICITY

Comment 1
No questions

Comment 2
I agree with the applicant's conclusion that there is no indication for an increased risk of adjuvant activity.

A.6. NUTRITIONAL ASSESSMENT

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

Comment 1
There is no reason to assume that the genetic modification has affected the nutritional value of food derived from MON 87427 × MON 89034 × NK603 maize.

Comment 2
No questions

Comment 3
The information provided in the application is sufficient.

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

Comment 1
There is no reason to assume that the genetic modification has affected the nutritional value of feed derived from MON 87427 × MON 89034 × NK603 maize.

Comment 2
No questions

Comment 3
The information provided in the application is sufficient.

B. EXPOSURE ASSESSMENT - ANTICIPATED INTAKE/EXTENT OF USE

Comment 1
No questions or comments. I fully agree with the conclusions.

Comment 2
P.122: 3.4. Conclusion, 2nd paragraph, last sentence: obviously, something is missing in this paragraph with regard to the intake of CP4 EPSPS, Cry1A.105 and Cry2Ab2 proteins in livestock diets.

Comment 3
The information provided in the application is sufficient.

C. RISK CHARACTERISATION

Comment 1
No questions.

Comment 2
The information provided in the application is sufficient.

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

Comment 1
No questions.

Comment 2
The information provided in the application is sufficient.

E. ENVIRONMENTAL RISK ASSESSMENT

E.1. INTRODUCTION

Comment 1
No questions

Comment 2
The information provided in the application is sufficient.

E.2. General approach of the ERA

Comment 1
Herbicide use in the USA on soybean, corn and cotton declined slightly in the first years following introduction of herbicide resistant GM crops, but increased moderately in recent years (Fernandez-Cornejo et al., 2014), whereas Benbrook (2012) reported that herbicide-resistant crop technology has led to a 239 million kg increase in herbicide use in the USA between 1996 and 2011.
MON 87427 × MON 89034 × NK603 maize is not intended for cultivation in the EU. Nevertheless, it may have consequences in countries were cultivation of MON 87427 × MON 89034 × NK603 maize is allowed. The continued application of the same herbicide in subsequent rotations may lead to increased selection pressure for herbicide resistant weed populations. Furthermore, the continued application of glyphosate may result in an increased accumulation of glyphosate in plant tissues (Bøhn et al., 2014; Rubio et al., 2014). Health concerns with regard to the use of glyphosate and its metabolites have been reported recently: Garry et al., 2002; Gasnier et al., 2009; George et al., 2010; Carman et al., 2013; Samsel en Seneff, 2013; Zouaoui et al., 2013; Guilherme et al., 2014; Krüger et al., 2014; Mesnage et al., 2014; Ackermann et al., 2015; Guyton et al., 2015; Seneff et al., 2015. Food and feed that compromises human and animal health is unacceptable. Therefore, the application doses of glyphosate in weed management should be rigorously respected. These considerations emphasize the importance of an appropriate weed management.
The applicant has referred to the characteristics that might potentially cause an adverse effect: the glyphosatetolerance, conferred by the CP4 EPSPS protein: see P.143, 1st paragraph.

SBB Comment:
The assessment of the use and safety of pesticides is not within the remits of the Biosafety Advisory Council.

Comment 2
No questions

Comment 3
The information provided in the application is sufficient.

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 or comments

Comment 2
The information provided in the application is sufficient.

E.3.2. PLANT TO MICRO-ORGANISMS GENE TRANSFER

Comment 1
No questions or comments. The conclusion is that “unlikelihood x unlikelihood x unlikelihood = unlikely”

Comment 2
The information provided in the application is sufficient.

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

Comment 1
No questions or comments. Not applicable or relevant for this submission

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

Comment 1
No questions or comments; (again, unlikehood x unlikehood x unlikehood = negligible).

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

Comment 1
Not applicable.

Comment 2
Not applicable

E.3.6. EFFECTS ON BIOGEOCHEMICAL PROCESSES

Comment 1
No questions or comments (same as E.3.4.).

Comment 2
The information provided in the application is sufficient.

E.3.7. EFFECTS ON HUMAN AND ANIMAL HEALTH

Comment 1
No new food or feed safety concerns are expected when stacked transgenes are not expressed in the same tissues or when their products are not translocated to the same tissues (Steiner et al., 2013).

Comment 2
No questions or comments.

Comment 3
The information provided in the application is sufficient.

E.3.8. Overall risk evaluation and conclusions

Comment 1
No questions or comments.

Comment 2
The information provided in the application is sufficient.

E.4. Post Market Environmental Monitoring plan

E.4.1. Interplay between environmental risk assessment and monitoring

Comment 1
No questions or comments.

Comment 2
The information provided in the application is sufficient.

E.4.2. Case-specific GM plant monitoring

Comment 1
No questions or comments.

Comment 2
The information provided in the application is sufficient.

E.4.3. General surveillance for unanticipated adverse effects

Comment 1
No questions or comments.

Comment 2
The information provided in the application is sufficient.

E.4.4. Reporting the results of monitoring

Comment 1
No questions or comments.

Comment 2
The information provided in the application is sufficient.

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