Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on application EFSA-GMO-NL-2016-134 (genetically modified maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 and its sub-combinations) from Monsanto under Regulation (EC) No. 1829/2003

17 September 2019
Ref. SC/1510/BAC/2019_0746

Context

Application EFSA-GMO-NL-2016-134 was submitted by Monsanto for the marketing of genetically modified (GM) maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 (Unique Identifier MON-87427-7 x MON-8746Ø-4 x MON-89Ø34-3 x SYN-IR162-4 x MON-ØØ6Ø3-6) and all of 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 87427 × MON 87460 × MON 89034 × MIR162 × NK603 was obtained by conventional crossing (no new genetic modification involved) of the corresponding single events:
- MON 87427, expressing the CP4 EPSPS protein that confers tolerance to herbicide products containing glyphosate;
- MON 87460, expressing the cold shock protein B (CSPB) for abiotic stress resistance, and the NPTII protein, a selectable marker and antibiotic resistance gene;
- MON 89034, expressing the Cry1A.105 and Cry2Ab2 proteins for resistance to lepidopteran insect pests;
- MIR162, expressing the Vip3Aa20 protein, conferring resistance to certain lepidopteran insect pests, and the PMI protein, a selectable marker;
- NK603, expressing the CP4 EPSPS protein and its variant CP4 EPSPS L214P, that confer tolerance to herbicide products containing glyphosate.

The application was validated by EFSA on 19 January 2017. A formal three-month consultation period of the Member States was started, in accordance with Articles 6.4 and 18.4 of Regulation (EC) No. 1829/2003 (consultation of national Competent Authorities within the meaning of Directive 2001/18/EC designated by each Member State in the case of genetically modified organisms being part of the products).

Within the framework of this consultation, the Belgian Biosafety Advisory Council (BAC), under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier, chosen from the common list of experts drawn up by the BAC and the Service Biosafety and Biotechnology (SBB). Nine experts answered positively to this request, and formulated a number of comments to the dossier. See Annex I for an overview of all the comments and the comments forwarded to EFSA.

The opinion of the EFSA Scientific Panel on GMOs was published on 8 August 2019 (EFSA Journal 2019;17(8):5774 [2]), toghether with the responses from the EFSA GMO Panel to comments submitted by the Member States during the three-month consultation period. On 12 August 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.2019.5774

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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-2016-134;
- The opinion of EFSA;
- The advices already adopted by the BAC on the single events and the lower-order stacks, which were as follows:

Event	Application number	BAC advice	Conclusions
MON 87427	EFSA-GMO-BE-2012-110	BAC/2015/0585 (08/09/2015)	Unlikely to pose any risk to human and animal health. No risk identified for the European environment.
MON 87460	EFSA-GMO-NL-2009-70	BAC/2013/0194 (25/03/2013)	Unlikely to pose any risk to human and animal health. No risk identified for the European environment.
MON 89034	EFSA-GMO-NL-2007-37	BAC/2009/0880 (03/02/2009)	No major risks for human and animal health or for the environment.
MIR162	EFSA-GMO-DE-2010-82	BAC/2012/0785 (29/08/2012)	No major risks for animal health or for the environment, no conclusion on human health. The PMI protein has been positively assessed in subsequent applications.
NK603	EFSA-GMO-NL-2005-22	BAC/2009/1367 (02/10/2009)	No major risks for human and animal health or for the environment.
MON 89034 x NK603	EFSA-GMO-NL-2007-38	BAC/2009/1492 (06/11/2009)	No major risks for human and animal health or for the environment.
MON87427 x MON89034 x NK603 and subcombinations	EFSA-GMO-BE-2013-117	BAC/2017/0741 (19/09/2017)	Unlikely to pose any risk to human and animal health. No risk identified for the European environment.
MON 87427 × MON 89034 × MIR162 × NK603 and subcombinations	EFSA-GMO-NL-2016-131	BAC/2019/0745 (17/09/2019)	Unlikely to pose any risk to human and animal health. No risk identified for the European environment.

All GM maize events mentioned in the table above, except for MON 87427 × MON 89034 × MIR162 × NK603, 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 87427 × MON 87460 × MON 89034 × MIR162 × NK603 (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 87427 × MON 87460 × MON 89034 × MIR162 × 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, CP4 EPSPS L214P, CSPB, NPTII, Cry1A.105, Cry2Ab2, Vip3Aa20 and PMI 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 CP4 EPSPS, CP4 EPSPS L214P, CSPB, NPTII, Cry1A.105, Cry2Ab2, Vip3Aa20 and PMI 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 × MON 87460 × MON 89034 × MIR162 × 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 whole set of data on maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 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 stacks, the Biosafety Advisory Council:

1) Agrees with the GMO panel of EFSA that the potential environmental release of maize MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 and its subcombinations 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 87427 × MON 87460 × MON 89034 × MIR162 × NK603 and its subcombinations are 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 stacks that have been assessed previously (see table on page 2) remain unchanged.

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-2016-134 and Comments submitted on the EFSAnet on mandate of the Biosafety Council (ref. BAC_2017_0263)

Bioveiligheidsraad
Conseil de Biosécurité

27 April 2017

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC_2017_0263
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Compilation of comments of experts in charge of evaluating the application EFSA/GMO/NL/2016/134
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 2 February 2017.
Coordinator: Dr. 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),

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/2016/134** concerns an application submitted by the company **Dow Agrosciences LLC** for authorisation to place on the market genetically modified **maize MON 87427 × MON 87460 × MON 89034 × MIR162 ×NK603** 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 27 January 2017.

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 comments

Comment 2
No comments

Comment 3
Based on its genetic properties MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 maize may be as safe for human and animal health and the environment as conventional maize based on the results of the compositional analysis and the weight of evidence with regard to the toxicological and the allergenicity assessment.
An adverse side-effect of this stacked event is that it may increase the use of glyphosate. Although the safety of glyphosate is not within the remits of BAC, a holistic approach of herbicide-tolerant GM crops seems desirable. Therefore, the approval may be postponed until new epidemiological and toxicology studies clearly demonstrate the safety of glyphosate and its metabolites for human and animal health and the environment (see E.3.8.).

Comment 4
MON 87427 x MON 87460 x MON 89034 x MIR162 x NK603 maize will be further referred to as 134 maize.

Comment 5
No comments

A. HAZARD IDENTIFICATION AND CHARACTERISATION

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

Comment 1
No questions

Comment 2
No comments

Comment 3
Evaluated, no comments

Comment 4
No comments

A.2. MOLECULAR CHARACTERISATION

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

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

Comment 1

Not applicable, because traditional breeding methods were used for establishing the stacked event as explained (see fig 1, p17)

Comment 2

No comments

Comment 3

The dossier describes the stacking of approved events.
No comments.

Comment 4

No comments

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 stacked event is expressing CP4 EPSPS providing tolerance to glyphosate like in MON87427
The stacked event is expressing CspB providing reduced yield loss under water limitation, hence drought resistance, as well as resistance to kanamycin as a selectable marker, as in MON87460
The stacked event is expressing CryA.105 and Cry2Ab2 proteins providing protection to certain lepidopteran pests as in MON89034
The stacked event is expressing Vip3Aa20 providing protection against some lepidopteran pests and PMI protein as a selectable marker as in MIR162
The stacked event is expressing CP4 EPSPS and CP4 EPSPS L214P providing tolerance to glyphosate like in NK603

Why these different crossings with MON87427 and NK603 for tolerance to glyphosate are needed? What is the added value of multiple expressing of these EPSPS? Or is this not the case since there is no evidence of interaction between the multiple copies of the cp4-epsps gene as explained on p 36-37; but again , why then the inclusion of multiple copies?

-under 1.2.3. (p35) it is mentioned that the inherited traits (glyphosate tolerance, drought tolerance and insect protection) are not expected to alter ….. survivability characteristics of maize!!
However, drought tolerance could increase survivability of maize in dry regions, when seed is accidently spilled, but very unlikely for other reasons as mentioned.
But it is a bit strange as formulated here that inherited traits (such as drought tolerance) are not expected to alter survivability characteristics of maize, while it is just the purpose of this GM-maize

expressing CspB-protein to provide reduced yield loss under water-limited conditions, hence to increase its survivability under water restricted conditions, or not ?

Comment from the coordinator :

This section refers to survival of maize without human intervention and/or in natural habitats.See also "Consensus Document on the Biology of Zea mays subsp. mays (Maize), OECD, 2003) "Maize has lost the ability to survive in the wild due to its long process of domestication, and needs human intervention to disseminate its seed. ..... however, maize is incapable of sustained reproduction outside of domestic cultivation. Maize plants are non-invasive in natural habitats ..... In contrast to weedy plants, maize has a pistillate inflorescence (ear) with a cob enclosed with husks. Consequently seed dispersal of individual kernels does not occur naturally."
The applicant argues that the transgenic traits will not modify survivability in natural habitats.

Comment 2
No comments

Comment 3
1.Out of all bioinformatic analyses, Kessenich and Silvanovich 2016c point to a similarity of the MON87460 insert with Ragweed homologues of the allergen Art v 1 (42.129% identity in a window of 83 amino acids with an E-score of 8.8e-7, which is above the threshold; page 12 of the quoted reference). However, the report presents sufficient arguments indicating that the similarity should have no biological significance, taking into account the location of the homology within the insert and the amino-acid composition bias in the aligned sequences. I support the arguments of the applicant.

2.In this dossier, integrity of the inserts in the stack as compared to the single events is shown by the sequence analysis of each event in the stack (required by the updated bioinformatic analysis), as reported in Vest et al. (2016). No Southern blot analysis is provided comparing the stack and the single events side by side. However, genetic stability of the events is inferred from the data obtained in the single events (cfr page 21 of the main dossier) and from the complex breeding tree indicating the successful inheritance of each event throughout the sexual generations leading to the commercialized stack. A theoretical discussion on why no mitotic or meiotic recombination is expected between the common sequences in the inserts is presented in section 1.2.2.4 (page 33 of main dossier). Altogether, the empirical ad theoretical elements provided by the applicant are sufficient to conclude on the integrity and on the stability of the events combined on the stack.

Comment 4
No comments

Comment 5
No comments

A.3. COMPARATIVE ASSESSMENT

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

Comment 1
LH244xLH287 as the conventional counterpart has the similar genetic background as the stacked product; 18 commercial reference varieties were used in the comparative assessments to provide a

range of comparative values representative for the natural variability within commercial maize varieties.

Comment 2
As it is usually the case 134 maize was compared to a conventional counterpart with similar background genetics as 134 maize and other commercially available maize hybrids.

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

Comment 1
No questions or comments

Comment 2
No remark

A.3.3. COMPOSITIONAL ANALYSIS

Comment 1
No questions; no compositional changes relevant from a food or feed safety perspective (cfr. Table 9 and 10, p42-43)

Comment 2
MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 maize is compositionally similar to the conventional maize comparator.
No residue concentrations were given for glyphosate in case of MON 87427 × MON 89034 × MIR162 × NK603 maize treated with glyphosate. High residue concentrations may inhibit rumen digestion, as shown by Reuter et al. (2007), using an *in vitro* experiment.

Comment 3
Compounds were selected according to the OECD guidelines.
No doubt this approach is suitable for a comparative assessment.
As a source of information is not adequate according to the actual insights in human nutrition.

I want to reiterate some comments I made already in previous evaluations of similar applications:

Total dietary fibre is assessed in addition to ADF and BDF; this is an important step forwards.
Carbohydrates are calculated by difference; a further differentiation of carbohydrates is desirable in further applications.

In the area of vitamins vitamin E or α-tocopherol is studied. No information is available for the other tocopherols(β, γ and δ) and the equivalent tocotrienols. Maize oil is a highly polyunsaturated oil, with excellent nutritive properties, but sensitive to oxidation. Tocopherols and tocotrienols have good antioxidative properties and stabilize the oil in a natural way. Any modification in the amount of these antioxidants could affect the stability, or in this case the safety , of the oil.

Also in the area of vitamins vitamin A is mentioned. In this case this means provitamin A or β-carotene. There are however some other carotenoids present in maize such as lutein and zeaxanthin. As the name zeaxanthin suggests it is a compound typical for maize. No information is available for these two compounds important in human nutrition.

Results are statistically evaluated, presented in tables and discussed according to the EFSA guidelines.
It is concluded that differences observed are of no compositional relevance.
As mentioned above this conclusion is based upon the compounds studied.

I agree with the overall conclusion.

Comment 4
Nearly all values for the anti-nutrients and secondary metabolites are significantly lower than those in its conventional counterpart, but are equivalent to the conventional commercial reference hybrids. No problem arises.

A.3.4. AGRONOMIC AND PHENOTYPIC CHARACTERISTICS

Comment 1
No questions

Comment 2
I found no information about the presence of mycotoxins. Maize is known to be one of the most sensitive grains, subject to mold infection during the growing season and the potential presence of a series of mycotoxins.

A.3.5. EFFECTS OF PROCESSING

Comment 1
No questions

Comment 2
Taking into account that 134 maize is not different from conventional maize no particular effects on processing are to be expected.
I agree with this conclusion.

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
The safety for humans and animals of the CP4-EPSPS, CspB, NptII, CryA.105,Cry2Ab2,Vip3Aa20,PMI-proteins has been demonstrated before, and is based on molecular characterization, history of safe use, lack of structural similarities with known toxins or allergens, rapid digestion in simulated gastric fluid. The proteins newly expressed in the stacked event are identical, no other biologically relevant differences are identified between the stacked event and its conventional counter part; therefore no need or added value in 90-day feeding studies in rats to assess food or feed safety; it is even not scientifically justified (as mentioned on p52 of Part II of scientific information).

Comment 2
Evaluated, no comments

Comment 3
The chance that the new proteins of (CP4 EPSPS, CP4 EPSPS L214P, CspB, Cry1A.105, Cry2Ab2, Vip3Aa20 and PMI) will pose serious risks for toxicity is negligible, based on the biochemical characterization of the newly expressed protein, the bioinformatics analysis that uses sequence searches to identify any similarities to toxins and anti-nutrients, inactivation of new proteins during heat processing and the in-vitro protein stability. 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 MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 maize (Steiner et al., 2013).

Comment 4
As can be expected, the amount of CP4 EPSPS is higher in the stacked event than in MON 87427 or NK603 as such. Still large margins of exposure were calculated.
CP4 EPSPS, CspB, NptII, CrylA.105, Cry2Ab2, Vip3Aa20 and PMI proteins have been demonstrated to be rapidly digested in simulated digestive fluids.

28 Day Repeat Dose Toxicity Study by Oral Gavage in Rats.
Not performed. No further testing is needed.

Sequence homology with known toxins.
All of the studies were performed in 2016. No analogy with known toxins was found.

A.4.3. ASSESSMENT OF NEW CONSTITUENTS OTHER THAN PROTEINS

Comment 1
Not relevant

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

Comment 1
No questions

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

Comment 1
No questions, see remarks under A.4.2

Comment 2

90-Day rat feeding study.
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
No questions

Comment 2
Evaluated, no comments

Comment 3
The lack of allergenic potential of the newly expressed proteins has been addressed individually in previous EFSA applications. An updated bioinformatics analysis for amino acid sequence homology with known allergens has been performed by the applicants using the Allergen AD_2016 database. No relevant sequence homologies were observed.
The specificity of the CP4 EPSPS, CspB, NptII, CrylA.105, Cry2Ab2,Vip3Aa20 and PMI proteins make it unlikely that in the stacked event, the proteins would have synergistic or antagonistic effects to each other or modify each other chemically resulting in allergenicity. Maize being a segregating crop, this is especially so for the grain where the individual events segregate, preventing interaction between the introduced proteins. Accordingly, I agree with the applicant's conclusion that no concerns in relation to allergenicity of the (stacked) newly expressed proteins were identified.

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

Comment 1
No questions

Comment 2

MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 maize seems to be comparable with conventional maize with regard to allergenicity, and it is unlikely that it will pose a serious allergenic risk.

Comment 3

I agree with the applicant that at the level of the whole plant no indications of potential adverse effects are identified. Yet, I **do not agree** with the applicant's proposition that on this basis a 90-day feeding study with whole food and feed in rodents is not justified. Considering the multiplicity of introduced events, the multiple pathways that may be affected and the fact that biological pathways are intricate, often interconnected and therefore not always behave as expected, such a feeding study provides in my opinion the best evidence for safety of the stacked GM maize.
The strains expressing the individual events are already on the market for quite some time. What about monitoring reports? I looked for this info in the dossier but could not find a reference to health monitoring reports. If available (why not), these should be included.

A.5.3. ADJUVANTICITY

Comment 1
No questions

Comment 2
No comments

A.6. NUTRITIONAL ASSESSMENT

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

Comment 1
No questions; the stacked event is no different from the near isogenic control and equivalent to maize varieties in commerce; no nutritional imbalances can be expected therefor as a result of the presence of the stacked event in the supply of maize in feed or food.

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 87427 × MON 87460 × MON 89034 × MIR162 × NK603 maize based on the compositional equivalence.

B. EXPOSURE ASSESSMENT - ANTICIPATED INTAKE/EXTENT OF USE

Comment 1
Reasonable certainty that consumption of foods derived from the stacked event will not affect health of adults and children based on large margins of exposure (MOE's). These are calculated by dividing

NOAEL from acute mouse gavage studies by human dietary intake values of the newly expressed proteins (as mentioned in table 16) . All MOE's are at least a factor 1.000 to 1.000.000

C. RISK CHARACTERISATION

Comment 1
No questions

Comment 2
Evaluated, no comments

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

Comment 1
No questions

Comment 2
Evaluated, no comments

E. ENVIRONMENTAL RISK ASSESSMENT

E.1. INTRODUCTION

Comment 1
No comments

Comment 2
No comments

Comment 3
A side effect of the use of genetically modified MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 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.

Comment from the coordinator :

The study of Santos-Amaya *et al*. suggests there is cross resistance in *S. frugiperda* towards Cry1F and Cry1A.105, and that there is no cross-resistance to Vip3Aa20 that is also present in the stack. The study results may inform resistance management measures.

E.2. General approach of the ERA

Comment 1
No comments

Comment 2
No comments

E.3. Specific areas of risk

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

E.3.1. Persistence and invasiveness including plant-to-plant gene flow

Comment 1
No questions

Comment 2
No comments

Comment 3
Evaluated, no comments

E.3.2. Plant to micro-organisms gene transfer

Comment 1
No questions

Comment 2
No comments

Comment 3
On page 86 of the main dossier, the applicant states "*However, none of the genetic elements inserted into the parental lines and inherited in MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 have a genetic transfer function."* This is not true as in maize MON 87460, the nptII gene is flanked by

two *loxP* sites, being part of the bacteriophage P1 related site-specific recombination system. The possibility that P1-mediated recombination could enhance the probability of horizontal transfer of the loxP-nptII-loxP cassette to bacterial cells was analysed in detail by EFSA in its previous assessment of the single MON 87460 event and finally not considered as raising a concern (EFSA Journal 2012 10(11):2936).

I see no reason why the reasoning for the single event would not be still valid for the stack. However, the applicant should have reported on this and referred to the extensive discussion triggered by the presence of the *lox* sequences in MON 87460.

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

Comment 1
Not relevant

Comment 2
No comments

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

Comment 1
No questions

Comment 2
No comments

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

Comment 1
Not relevant

Comment 2
No comments

Comment 3
Evaluated, no comments

Comment 4
MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 maize is glyphosate tolerant, which may result in an increased application of glyphosate-based herbicides. High residue concentrations 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). Health concerns with regard to the use of glyphosate have been reported (Mensah et al., 2015).

E.3.6. EFFECTS ON BIOGEOCHEMICAL PROCESSES

Comment 1
Negligible

Comment 2
No comments

Comment 3
Evaluated, no comments

E.3.7. EFFECTS ON HUMAN AND ANIMAL HEALTH

Comment 1
See earlier (under A); no further questions

Comment 2
No comments

Comment 3
The new proteins in MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 maize are unlikely to be detrimental for human and animal health. However, there may be an adverse side effect of the use of MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 maize: glyphosate residues and its metabolite may be harmful for human and animal health.

E.3.8. OVERALL RISK EVALUATION AND CONCLUSIONS

Comment 1
No comments

Comment 2
No comments

Comment 3
Evaluated, no comments

Comment 4
Because of the controversy between the WHO and EFSA with regard to the safety of glyphosate (EFSA, 2015; Guyton et al., 2015; Portier et al., 2016) a new examination of glyphosate toxicity should be undertaken to adjust downward the acceptable daily intake for glyphosate, as proposed by Myers et al. (2016). Furthermore, the European Chemicals Agency is conducting an investigation into the wider human health effects of glyphosate: see http://echa.europa.eu/registry-of-submitted-harmonised-classification-and-labelling-intentions/-/substance-rev/13201/term. In the meantime, the approval of MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 maize may be postponed.

E.4. POST MARKET ENVIRONMENTAL MONITORING PLAN

E.4.1. INTERPLAY BETWEEN ENVIRONMENTAL RISK ASSESSMENT AND MONITORING

Comment 1
No questions

Comment 2
Evaluated, no comments

E.4.2. CASE-SPECIFIC GM PLANT MONITORING

Comment 1
No questions

Comment 2
Evaluated, no comments

E.4.3. GENERAL SURVEILLANCE FOR UNANTICIPATED ADVERSE EFFECTS

Comment 1
No questions

Comment 2
Evaluated, no comments

E.4.4. REPORTING THE RESULTS OF MONITORING

Comment 1
No questions

Comment 2
Evaluated, no comments

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