Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on application EFSA-GMO-NL-2015-127 (genetically modified maize 1507 x MON810 x MIR162 x NK603 and its subcombinations) from Pioneer under Regulation (EC) No. 1829/2003

22 January 2021
Ref. SC/1510/BAC/2021_0068

Context

Application EFSA-GMO-NL-2015-127 was submitted by Pioneer for the marketing of genetically modified (GM) maize 1507 x MON810 x MIR162 x NK603 (Unique Identifier DAS-Ø15Ø7-1 x MON-ØØ81Ø-6 x SYNIR162-4 x MON-ØØ6Ø3-6) and its subcombinations, 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 1507 x MON810 x MIR162 x NK603 was obtained by conventional crossing (no new genetic modification involved) of the corresponding single events:
- 1507, expressing the Cry1F and PAT proteins, conferring resistance to certain lepidopteran pests and tolerance to herbicide products containing glufosinate ammonium;
- MON810, expressing the Cry1Ab protein that confers resistance to certain lepidopteran pests;
- MIR162, expressing the Vip3Aa20 protein that confers resistance against specific lepidopteran insects and the gene of phosphomannose isomerase (PMI) serving as selection marker;
- NK603, expressing the CP4 EPSPS and its variant CP4 EPSPS L214P proteins for tolerance to glyphosate-containing herbicides.

The application was validated by EFSA on 9 February 2016. A formal three-month consultation period of the Member States was started, lasting until 10 May 2016, 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 on 9 May 2016.

The opinion of the EFSA Scientific Panel on GMOs was published on 13 January 2021 (EFSA Journal 2021;19(1):6348[2]), together with the responses from the EFSA GMO Panel to comments submitted by the Member States during the three-month consultation period. On 14 January 2021 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.2021.6348

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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-2015-127;
- The opinion of EFSA;
- The advices already adopted by the BAC on the single events and lower-order stacks. The conclusions of the BAC for the most recent applications were as follows:

Event	Application number (EFSA-GMO-)	BAC advice	Conclusions
1507	RX-001	BAC/2017/0186 (21/03/2017)	No major risks for human and animal health or concerning the environment were identified. (minority declaration related to the lack of statistically convincing studies on toxicity)
MON810	RX-MON810	BAC/2009/01510 (17/11/2009)	No major risks for human and animal health or concerning the environment were identified.
MIR162	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	NL-2005-22	BAC/2009/1367 (02/10/2009)	No major risks for human and animal health or concerning the environment were identified.
MON810 x NK603	RX-007	BAC/2018/0215 (17/04/2018)	No major risks for human and animal health or concerning the environment were identified.
1507 x NK603	RX-008	BAC/2018/0705 (11/09/2018)	No major risks for human and animal health or concerning the environment were identified.
1507 x MIR162	DE-2011-103	BAC/2019/0393 (13/05/2019)	Unlikely to pose any risk to human and animal health. No risk identified for the European environment.
MIR162 x NK603	NL-2016-134	BAC/2019/0746 (17/09/2019)	Unlikely to pose any risk to human and animal health. No risk identified for the European environment.
1507 x MON810 x NK603 and subcombinations	NL-2011-92	BAC/2018/0055 (30/01/2018)	Unlikely to pose any risk to human and animal health. No risk identified for the European environment.

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

[3] See EU register of GM food and feed: http://ec.europa.eu/food/dyna/gm_register/index_en.cfm

Scientific evaluation

1. Environmental risk assessment

The Biosafety Advisory Council is of the opinion that it is unlikely that the accidental release of maize 1507 x MON810 x MIR162 x 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. 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 1507 x MON810 x MIR162 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 Cry1F, PAT, Cry1Ab, Vip3Aa20, PMI, CP4 EPSPS and CP4 EPSPS L214P 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 Cry1F, PAT, Cry1Ab, Vip3Aa20, PMI, CP4 EPSPS and CP4 EPSPS L214P 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 1507 x MON810 x MIR162 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.

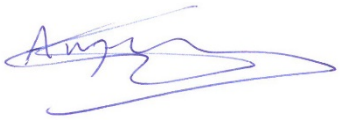
[4] As the application doesn't imply cultivation of the GM crop in the EU, a full environmental assessment, as in the case of cultivation dossier, is not warranted.

Conclusion

Based on the whole set of data on maize 1507 x MON810 x MIR162 x 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 four single events and some lower-order stacks, the Biosafety Advisory Council:

1) Agrees with the GMO panel of EFSA that the potential environmental release of maize 1507 x MON810 x MIR162 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 1507 x MON810 x MIR162 x NK603 is unlikely to pose any risk to human and animal health;

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



Dr. ir. Geert Angenon
President of the Belgian Biosafety Advisory Council

Annex I: Compilation of comments of experts in charge of evaluating the application EFSA-GMO-NL-2015-127 and comments submitted to EFSA on mandate of the Biosafety Council (ref. BAC_2016_0250)

Bioveiligheidsraad
Conseil de Biosécurité

09/05/2016

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC_2016_0250
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Compilation of comments of experts in charge of evaluating the application EFSA/GMO/NL/2015/127
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 23 February 2016.
Coordinator: Geert Angenon
Experts: Eddy Decuypere (KUL), Jacques Dommes (ULg), Patrick du Jardin (ULg), Leo Fiems (ILVO), Johan Grooten (UGent), André Huyghebaert (UGent), Peter Smet (Consultant), Frank Van Breusegem (UGent), Jan Van Doorsselaere (KATO), 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/2015/127** concerns an application submitted by the company **Pioneer** for authorisation to place on the market genetically modified maize **1507 x MON810 x MIR162 x NK603** and all sub-combinations 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 9th February 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
The 4-stacked event 1507 x MON810 x MIR162 x NK603 maize was evaluated as a whole, meaning that possible repercussions of the genetically modified maize were taken into account, not only because of the presence of new proteins, but also because it may have implications for human and animal health by the presence of residues of the herbicides itself or their metabolites.

Single events dealing with CP4 EPSPS, Cry1Ab, Cry1F, PAT PMI and Vip3Aa20 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 1507 x MON810 x MIR162 x NK603 maize is no reason to prohibit its import and processing in the EU.

Although there is no direct effect of the genetic modification of 1507 x MON810 x MIR162 x NK603 maize, an indirect effect cannot be excluded due to an increased use of glyphosate and glufosinate. Some health concerns about glyphosate have been reported. 1507 x MON810 x MIR162 x NK603 maize is not intended for cultivation in the EU. The introduction of the 1507 x MON810 x MIR162 x NK603 maize elsewhere in the world may increase the use of these herbicides. As a consequence, imported maize, destined for food and feed use, may contain residues of these herbicides and their metabolites.

It is advised that the EU should delay the approval of the import of 1507 x MON810 x MIR162 x NK603 maize until new epidemiological and toxicology studies clearly demonstrate the safety of glyphosate and its metabolites for human and animal health and the environment.

SBB Comment:
The assessment of the safety of pesticides/herbicides is not within the remits of the BAC.

Comment 3
None.

Comment 4
No comments.

A. HAZARD IDENTIFICATION AND CHARACTERISATION

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

Comment 1
No comments.

Comment 2
No comment, adequate information was provided.

Comment 3
None.

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
1507 x MON810 x MIR162 x NK603 is obtained by traditional crossing between modified 1507, Mon810, etc. and the single events behave as independent loci.
F2 is produced from selfed hemizygous hybrid F1 seeds [1507 x MON810 x MIR162 x NK603].

Comment 2
No comment, adequate information was provided.

Comment 3
No comments.
The dossier concerns the stacking of four events which have been approved by EFSA. It is shown that in Maize 1507 x MON810 x MIR162 x NK603 the stability of the inserts is maintained and the expression of the proteins is as in the single events.

Comment 4
- Page 26 of main dossier and Annex 4 (Table 1.). BLASTp analysis of the Cry1F protein returns hits with two uncharacterized proteins [*Fusarium fujikuroi* IMI 58289], with E-values of 8.00E-06—0.006. No further information is given and the report concludes by saying: « BLASTp search (updated January 18, 2015) of the Cry1F protein generated an output file with more than 600 pages. The BLASTp search output file is digitally stored in the Dow AgroSciences archive.»
Considering that Fusarium species are known to produce toxins and that the E-value is not so high, the notifier should display the output alignments in the dossier and make further comments.
- Page 26 of main dossier and Annex 3. In the search for similarities between PAT and toxins, the applicant discusses hits with GNAT toxin components and refers to « Appendix 1 in a separate pdf file » which I could not find. As a matter of principle, all reports referred to by the dossier should be made available to experts. In the present case, figures displaying the main alignments of concern are given in annex 3, which I consider as sufficient.

SBB Comment:
The mentioned BLAST output files were provided in the dossier but overlooked by the expert.

Coordinator Comment:

The blast output files are indeed provided. *Fusarium* mycotoxins are in general produced through complex pathways by non-ribosomal peptide synthetases; therefore, the short stretches of homology found in a single gene do not raise concern.

- Page 26 and Annex 7 (pages 9 and 13, and later): the toxin similarity search used as 'threshold of significance' an E value of 1X10-5. Could the applicant justify why? Such threshold of significance is not adopted for some other expressed proteins within the same dossier (see e.g. Cry1F) and the rationale for such discrepancies should be given. I suspect this is just the consequence of the contrasting approaches by the different companies owning the different single events... Whether current EFSA guidelines for bioinformatic analyses are followed would be worth checking.

SBB and Coordinator Comment
The phrase "This highlights the need for harmonisation in the reporting of similarity searches." was added to the above comments highlighted in grey for posting on EFSAnet.

Comment 5
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
No biologically relevant changes in protein expression values were observed between 1507 x Mon810 x Mir162 x NK603 maize and in single event maize lines. Therefore, no interactions at the DNA and RNA level influencing protein expression levels can be observed in the crossed events compared to single event lines. This conclusion is supported by conclusions on other crossings events such as 1507 x NK603 (EFSA2006) and NK603 x Mon810 (EFSA2005).
The characterization and risk assessments of each of the single events and the higher order stack (1507 x Mon810 x Mir162 x NK603) maize are scientifically relevant to cover risk assessment of all the sub-combinations independently of their origin.

Comment 2
No comment, adequate information was provided.

Comment 3
Although there is no evidence of any instability of the transgenes in 1507 x MON810 x MIR162 x NK603 maize (Technical Dossier, P.56) some alertness is desirable. Ali et al. (2014) assumed that stacked events tend to be more instable than single events, and MON810 maize was a parental GM line used for the breeding of 1507 x MON810 x MIR162 x NK603 maize.

Coordinator comment:
The evidence for this conclusion in the paper by Ben-Ali et al. (2014) is very weak; the broader significance is not clear, so far.

Comment 4

- page 50 of main dossier and Annex 19 (page 8): for the MIR162 event, bioinformatic analysis of the putative translation products of the ORFs (insert + junctions) using the BLASTP algorithm uses E-cut-off values of 1X10e-6 and 1X10e-5 for allergens and toxins respectively. Again (see previous remark under A.2.1), this is not fully consistent with the bioinformatic searches for the other events and is not justified by the applicant (compliance with guidelines?).
- Protein expression (table 11 page 52 of main dossier): for Vip3Aa20, a two-fold difference in the mean value is noticed when comparing the intended vs conventional herbicide treatments in the 4-event stack. No comment is made by the applicant. Is it possible that this difference is caused by the herbicide regime? Was this already observed with previously assessed subcombinations? The applicant should comment this difference in protein levels.

General conclusion on the MC part: no hazards and risks have been identified by the MC but some shortcomings occur in the methodology and in the completeness of the data provided in the dossier by the applicant.

Comment 5
No comments.

A.3. COMPARATIVE ASSESSMENT

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

Comment 1
16 non-GM reference lines were used (3 per site) + a non GM control conventional counterpart with a genetic background as close as possible using a cross of 2 non-GM inbred lines.
CHT (with nicosulfuron, diflufenzopyr, and dicamba) on all control maize, references maize and the stacked event
IHT (with glyphosate and glufosinate) on the stacked event
No further questions.

Comment 2
None.

Comment 3
Maize 1507 x MON810 x MIR162 x NK603 is obtained by traditional breeding of genetically modified maize. As no new genetic modification is applied the risk of significant differences with traditional maize is rather low.
Non-GM near-isogenic maize is chosen as a comparator. As in other similar applications data from 16 commercial non-GM maize hybrids are also used in order to compare with the range of natural variations.

No further remarks.

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

Comment 1
OK

Comment 2
None.

Comment 3
The approach is in line with previous applications. Field trials were conducted in the 2012 growing seasons in the United States and Canada. A randomized complete block design was applied. Statistical analysis was done according to the OECD Guidelines.

No further remarks.

A.3.3. COMPOSITIONAL ANALYSIS

Comment 1
All of the 71 compounds analysed fell into outcome 1-4 and none were found in types 5-7, meaning that the centre of confidence interval is always inside EI. Most differences is type 4 difference between GM and isogenic control, for gamma – tocopherol, vitB1, crude fat, isoleucine, leucine and phenylalanine. However, differences were small and fell within the range of natural variation so that no biological relevance was attributed to the differences observed.
For gamma-tocopherol, levels of GM-maize were sometimes a bit higher than the control, but lower than the reference lines, pointing to a broad range of variability between different maize lines. I agree with the conclusion that no biological relevance has to be attributed to apparent differences for these vitamins (VitB1, B5 and tocopherol) in this study.
The conclusion that comparative analysis of 1507 x Mon810 x Mir162 x NK603 maize demonstrated the comparability to any other commercial maize and that no unintended effects resulting from the genetic modification have been observed, is valid.

Comment 2
Some compounds analysed in 1507 x MON810 x MIR162 x NK603 maize (fat, isoleucine, leucine, and phenylalanine, vitamin B1, vitamin B5, γ-tocopherol and inositol) were different from non-GM maize. However, mean values are within the range of maize references and the absolute differences between them are minor, so that differences are not biologically relevant.

The concentrations of glyphosate and glufosinate were not discussed in section 1.3.4. Comparative analysis of composition and Annex 22. As part of 1507 x MON810 x MIR162 x NK603 maize was treated with glyphosate and glufosinate (Technical Dossier, P.64), it is highly desirable to report the concentrations of glyphosate and its metabolites, and of glufosinate.

Comment 3 **(du Jardin)**
None.

Comment 4 **(Huyghebaert)**
Once again the OECD Guidelines were followed in the selection of compounds for comparative analysis.
Grain assessment included proximates, fibre, fatty acids, amino acids, minerals, vitamins, secondary metabolites and anti-nutrients.
Forage assessment included proximate, fibre and minerals.

Some comments on the selection of constituents in grain according to the OECD guidelines:
proximates
- detergent fibre approach
- carbohydrates: no further specification
- both approaches are not in agreement with Regulation 1169/2011 of the European Parliament and the Council on Food Information to Consumers, with amendments, OJ European Union of 22.11.2011 Under annex I Specific Definitions
Coordinator comment:
Correct, but this legislation is not relevant for risk assessment of GM plants

1. Nutrition Labeling
   -
   -
   -carbohydrates (sugars, polyols, starch)
   -
   -fibre: definition under 12

fatty acids
- no comments as all relevant fatty acids in maize are included and studied in detail: e.g. differentiation of linolenic acid and γ-linolenic acid
amino acids
- no comments as all essential (indispensable) amino acids are included
minerals
no comments
vitamins
- significant vitamins are included; tocopherols are differentiated into α, β, γ and ó- tocopherol but no results are given for the range of tocotrienols,
- with respect to pro-vitamins results are given for β- carotene but not for relevant carotenoids in maize such as
xanthophylls.
secondary metabolites and anti-nutrients
- no further comments

Only a few analytes showed statistically significant differences or non-equivalence. However all data were in the range of natural variation.
Significant differences or lack of equivalence (type 2 and 4) were observed in grain for crude fat, 3 amino acids, 3 vitamins and for inositol.

The applicant concludes that, after evaluation of literature data and own observations, these observations are not biologically relevant.

I agree with this conclusion.

Comment 5
There seem to be no problems concerning the secondary metabolites and antinutrient composition.

A.3.4. AGRONOMIC AND PHENOTYPIC CHARACTERISTICS

Comment 1

No questions.

Comment 2
None.

Comment 3
During the trials at different locations in the key maize growing areas in North America no unexpected agronomic differences were observed.

A.3.5. EFFECTS OF PROCESSING

Comment 1
No questions.

Comment 2
The applicant concludes that maize 1507 x MON810 x MIR162 x NK603 is safe and nutritionally equivalent to food and feed products derived from conventional maize.

Taking into account the conclusions of the comparative study I agree with this conclusion.

I cannot identify a particular reason why a food or a feed product obtained by processing of maize 1507 x MON810 x MIR162 x NK603 would be different from traditional maize. This conclusion applies for the well-known wet and dry milling processes.

A.4. TOXICOLOGICAL ASSESSMENT

A.4.1. METHODOLOGY USED FOR TOXICITY TESTS

Comment 1
Besides the new proteins of the single events, there are no new proteins expressed in the stacked event.
The safety assessment of those proteins was realized before as listed on table 5.
The risk assessment of the stacked event is therefore logically focused on stability of the transformation events and potential synergistic or antagonistic effects resulting from the combination of the transformation events.
Since no evidence was found of any interactions between inserts at the level of gene transcription or translation, and also not between the insert-encoded proteins, no further testing for toxicology or for nutritional equivalence is needed. All single events were previously tested on toxicology and nutritional equivalence.

Comment 2
The application seeks authorisation for the placing on the market of GM 1507 x MON810 x MIR162 x NK603 maize for import, processing and all food and feed uses in accordance with Art.3(1) and 15(1) of regulation (EC) 182912003. IN ADDITION the application also seeks authorisation of the sub-combination of events, independently of their origin:

- 1507 x MON810 x MIR162

- 1507 x MIR162 x NK603
- MON810 x MIR162 x NK603
- MON810 x MIR162
- MIR162 x NK603

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
See A4.1.

Comment 2
It is unlikely that the new proteins of 1507 x MON810 x MIR162 x NK603 maize will pose serious risks for toxicity. It is assumed that there is no biological pathway in which the newly-inserted genes would directly or indirectly interact safety (Kok et al., 2014; Zdziarski et al., 2014). There is no plausible or testable hypothesis for the interaction of DMO and CP4 EPSPS proteins in 1507 x MON810 x MIR162 x NK603 maize (Steiner et al., 2013).

Comment 3
Page 91 of main text “New homology searches to known toxins”: Comments on the bioinformatic search for similarities with toxins have been done in the previous section A.2.1.

Comment 4
The amounts of the respective proteins in the stacked event are comparable to those in the single event lines.

Safety assessment of the different proteins was conducted earlier.

An up-to-date bioinformatics search was performed for each of the individual proteins and raised no concerns.

Comment 5
No new genetic modifications have been introduced in 1507 x MON810 x MIR162 x NK603 maize. The safety of the proteins Cry1F, Cry1Ab, Vip3Aa20, PMI, PAT, CP4 EPSPS has been confirmed in detail in accordance with the applications of authorisation of maize 1507 (and renewal), maize MON810 (and renewal), maize MIR162, and maize NK603 (and renewal). Maize 1507 x MON810 x MIR162 x NK603 was obtained by traditional crossing of the 4 GM single parental maize events. The inserts were all integrated into different loci in the maize nuclear genome. Updated bioinformatics evaluations were provided.
Expression levels of the insert-encoded proteins in 1507 x MON810 x MIR162 x NK603 maize were determined and were found comparable with the expression levels in the GM parental lines (field study, 2012 growing season, 4 sites, USA).
No further comments or questions.

A.4.3. ASSESSMENT OF NEW CONSTITUENTS OTHER THAN PROTEINS

Comment 1
Not relevant.

Comment 2
No further comments or questions.

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

Comment 1
No questions.

Comment 2
Compositional analysis of 1507 x MON810 x MIR162 x NK603 maize grain and forage.
The nutrient compositional analysis was performed on grain and forage of a field study with maize grown in one growing season (2012) in the USA and Canada at eight separate locations.
Statistics according EFSA guidelines using difference and equivalence testing.
Grain: Significant differences and/or lack of equivalences

Crude fat: CHT 2 (I), IHT 4 (II)
Amino acids: Isoleucine CHT 2 (I), IHT 4 (II); Leucine CHT 2 (I), IHT 4 (II); Phenylalanine CHT 2 (I), IHT 4 (II)
Vitamins: Vitamin B1 CHT 4 (II), IHT 3 (II); Vitamin B5 CHT 3 (II), IHT 3 (II); gamma-Tocopherol CHT 4 (II), IHT 4 (II)
Secondary metabolites: Inositol CHT 1 (I), IHT 3 (II)

Forage: ok
The differences fell within the reference range and the tolerance intervals. No further comments or questions.

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

Comment 1
No questions, see A4.1.

Comment 2
Not performed, no further testing is needed at the moment.

Comment 3
The applicant submitted a nutritional performance study, but no 90-day feeding toxicity study in rodents.

42-day poultry feeding study.
Groups: 1) Non transgenic near-isogenic control maize (091) grain: conventional herbicide-treated; 2) 1507 x MON810 x MIR162 x NK603 maize grain: conventional herbicide-treated; 3) 1507 x MON810 x MIR162 x NK603 maize grain: glyphosate/glufosinate treated, and 3 reference maize grains 4) 33N43,

5) P1236, 6) P1253.
No adverse effects could be detected.

Long-term impact on human and animal health
Some applications concerning stacked transformation events including MON810, NK603, 1507 and MIR162 have been assessed by EFSA. No medium-term feeding studies in rodents are made available for the stacked events
Adopted applications:

Application	Single or Stacked event	90-day rat feeding study	Broiler study
UK-2004-05	1507xNK603	No study	OK
UK-2004-01	NK603xMON810	No study	OK
RX-NK603 and NL-2005-22	NK603	♂, ♀: ↓ RBC, ↑ platelets, ↑ haemaglobin, ↑ hematocrit, ♂: ↑ liver weight, ↑ heart weight (kidney weight not included in the study)	♂, ♀combined: breast meat weight (kg), fat pad weight (kg, % of live weight, thigh meat moisture (%)
RX-MON810	MON810	♀: ↑ platelets ♀: ↓ MCHC ♀, ♂: ↓ albumin/globulin ratio	OK
RX-1507 and NL-2004-02	1507	♂, ♀: ↓ RBC, ↓ platelets, ↓ haemaglobin, ↓ hematocrit, ♂: ↓ kidney weight	OK
DE-2010-82	MIR162	Low dose ♀: ↓ plasma glucose ♂: ↑ RBC, ↑alkaline phosphatase, ↓ rel kidney weight High dose ♀: ↓ basophils ♂: ↑ activatedpartial thromboplastine time	OK

No further comments or questions.

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 comments.

Comment 2
Shortcomings in the bioinformatics searches regarding justification of significance thresholds have been commented in the previous section A.2.1.

Comment 3
Assessment of individual events
Based on the following:

- A weight of evidence analysis performed before for the individual newly expressed proteins concluded that the Cry1F, PAT, Cry1Ab, Vip3Aa20, PMI and CP4 EPSPS proteins are highly unlikely to be allergenic;
- The approved single GM events have now been part of the food supply for years without incident;
- An updated bioinformatics analysis using a 2015 database did not reveal sequence homologies with known allergens;

I agree with the applicant's conclusion that there is no new evidence indicating an increased risk for allergenicity of either inserted protein.

Assessment of stacked events
Based on the following:

- The single events behave as independent genetic loci, thus rendering unlikely mutual interactions at the genetic level, modulating gene expression and/or stability;
- No (toxicological) evidence was found of any interactions between Cry1F, PAT, Cry1Ab, Vip3Aa20, PMI and CP4 EPSPS proteins, indicating that the stacked events will not mutually amplify each other's expression/activity and hereby increase the likelihood of allergenicity;
- No adverse effects have been observed after mixing of individual GM plants expressing the proteins;
- The allergenic potential of several sub-stack combinations of the insert-related proteins has been assessed before by EFSA;

I agree with the applicant's conclusion that it is highly unlikely that the co-expression of Cry1F, PAT, Cry1Ab, Vip3Aa20, PMI and/or CP4 EPSPS proteins in 1507 x MON810 x MIR162 x NK603 maize or in any sub-combination of these events is unlikely to cause an allergic reaction in humans or animals.

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

Comment 1
No comments.

Comment 2
Maize is not a major allergenic food. Therefore, it is unlikely that the co-expression of the insert-related proteins in 1507 x MON810 x MIR162 x NK603 maize will increase the allergenicity of the GMO.

A.5.3. ADJUVANTICITY

Comment 1
No comments.

Comment 2
No elements are identified suggesting that combining Cry1F, PAT, Cry1Ab, Vip3Aa20, PMI and CP4 EPSPS proteins in the stacked GMO may increase the adjuvant potential of either protein, individually or combined.

A.6. NUTRITIONAL ASSESSMENT

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

Comment 1
No questions.

Comment 2
Based on the compositional equivalence and the fact that differences are not biologically relevant (see A.3.3), there is no reason to assume that the genetic modification has affected the nutritional value of food derived from 1507 x MON810 x MIR162 x NK603 maize.

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

Comment 1
Grain from non-modified isogenic control, reference hybrids and [1507 x MON810 x MIR162 x NK603] treated with conventional herbicides or with glyphosate and glufosinate were used.
The amount of maize used in the diets was high and no effects on performance parameters were reported. It is also of relevance that either CHT or IHT treated stacked events had no effect on performance. It is a pity that a summarizing table of all results + deviations and outliers is not given.

Comment 2
Based on the compositional equivalence and the fact that differences are not biologically relevant (see A.3.3), there is no reason to assume that the genetic modification has affected the nutritional value of feed derived from 1507 x MON810 x MIR162 x NK603 maize.

B. EXPOSURE ASSESSMENT - ANTICIPATED INTAKE/EXTENT OF USE

Comment 1
No questions.

C. RISK CHARACTERISATION

Comment 1
No questions.

Comment 2
None.

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

Comment 1
No questions.

Comment 2
None.

E. ENVIRONMENTAL RISK ASSESSMENT

E.1. INTRODUCTION

Comment 1
No questions.

Comment 2
No comment, adequate information was provided.

Comment 3
None.

E.2. GENERAL APPROACH OF THE ERA

Comment 1
No questions.

Comment 2
No comment, adequate information was provided.

Comment 3
None.

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; the conclusions that are formulated for the stacked event also apply to all its sub-combinations.

Comment 2
No comment, adequate information was provided.

Comment 3
None.

E.3.2. PLANT TO MICRO-ORGANISMS GENE TRANSFER

Comment 1
No questions.

Comment 2
No comment, adequate information was provided.

Comment 3
None.

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

Comment 1
Not relevant.

Comment 2
No comment, adequate information was provided.

Comment 3
None.

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

Comment 1
No questions.

Comment 2

No comment, adequate information was provided.

Comment 3
None.

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

Comment 1
Not applicable.

Comment 2
No comment, adequate information was provided.

Comment 3
1507 x MON810 x MIR162 x NK603 maize is not intended for cultivation in the EU. Nevertheless, the introduction of glyphosate-tolerant crops may result in the accumulation in soils of glyphosate and its metabolites (aminomethylphosphonic acid) in regions where its cultivation is allowed, so that the sustainability of genetically modified glyphosate-tolerant crops is questionable (Mamy et al., 2010; Mortensen et al., 2012).

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. Glyphosate use has risen almost 15-fold since genetically modified glyphosate-tolerant crops were introduced in 1996 (Benbrook, 2016).

1507 x MON810 x MIR162 x NK603 maize is not intended for cultivation in the EU. Nevertheless, an indirect effect of the approval of 1507 x MON810 x MIR162 x NK603 maize is that it may have consequences in countries where its cultivation 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 same herbicides may result in an increased accumulation of residues of herbicides and metabolites in plant tissues (Reddy et al.,2008; Bøhn et al., 2014) and surface water (VMM, 2015). Health concerns with regard to the use of glyphosate (Guyton et al., 2015; Seneff et al., 2015) and glufosinate (Laugeray et al., 2014) have been reported. Food and feed that compromise human and animal health is unacceptable.

The application of these herbicides in weed management should meet the restrictions specific to herbicide-treated crops. Herbicide mixing exposes weeds to multiple mechanisms of action, which may delay resistance evolution. However, herbicide mixtures are not a permanent solution to the problem of herbicide resistance, as they do not prevent it on the long run (Mortensen et al., 2012; Evans et al., 2015).

SBB Comment:
The assessment of the safety of pesticides/herbicides is not within the remits of the BAC.

Comment 4

None.

E.3.6. EFFECTS ON BIOGEOCHEMICAL PROCESSES

Comment 1
No questions.

Comment 2
No comment, adequate information was provided

Comment 3
None.

E.3.7. EFFECTS ON HUMAN AND ANIMAL HEALTH

Comment 1
No questions.

Comment 2
No comment, adequate information was provided.

Comment 3
The new proteins in 1507 x MON810 x MIR162 x NK603 maize are unlikely to be detrimental for human and animal health. However, there is a side effect of the use of 1507 x MON810 x MIR162 x NK603 maize: glyphosate residues and its metabolites and glufosinate residues may be harmful for human and animal health.

SBB Comment:
The assessment of the safety of pesticides/herbicides is not within the remits of the BAC.

Comment 4
None.

E.3.8. OVERALL RISK EVALUATION AND CONCLUSIONS

Comment 1
No questions.

Comment 2
No question. I agree with the conclusion that the intended uses of this genetically modified maize will not pose more risks to human and animal health or the environment than non-GM varieties.

Comment 3
Because of the controversy between the WHO (Guyton et al., 2015) and EFSA (EFSA, 2015) with regard to the safety of glyphosate, 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). In the meantime, the approval of 1507 x MON810 x MIR162 x NK603 maize for import and processing should be postponed.

Coordinator comment:
The assessment of the safety of pesticides/herbicides is not within the remit of the BAC.

Comment 4
None.

E.4. Post Market Environmental Monitoring plan

E.4.1. Interplay between environmental risk assessment and monitoring

Comment 1
No questions.

Comment 2
None.

E.4.2. Case-specific GM plant monitoring

Comment 1
No questions.

Comment 2
None.

E.4.3. General surveillance for unanticipated adverse effects

Comment 1
No questions.

Comment 2
None.

E.4.4. Reporting the results of monitoring

Comment 1
No questions.

Comment 2
None.

ADDENDUM FROM THE REVIEWING EXPERT : ADDITIONAL INFORMATION FROM THE SYSTEMATIC REVIEW OF THE LITERATURE

The dossier also reports on the systematic review of published studies pertaining to the safety of the stacked events (see page 147 &sq.) I read the conclusions of the applicant and have no comments.

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