Bioveiligheidsraad
Conseil de Biosécurité

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Secretariaat
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O./ref.: WIV-ISP/41/BAC/2017_0742

Advice of the Belgian Biosafety Advisory Council on application EFSA-GMO-BE-2013-118 from Monsanto Europe S.A./N.V. under Regulation (EC) No. 1829/2003

Context

Application EFSA-GMO-BE-2013-118 was submitted by Monsanto Europe S.A./N.V. on 26 November 2013 for the marketing of genetically modified (GM) maize MON87427 x MON89034 x 1507 x MON88017 x 59122 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 five-event stack maize MON87427 x MON89034 x 1507 x MON88017 x 59122 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;
- 1507, expressing the Cry1F protein for resistance to lepidopteran pests, and the PAT protein for tolerance to glufosinate-ammonium herbicides;
- MON88017, expressing the CP4 EPSPS and Cry3Bb1 proteins, for glyphosate tolerance and resistance to lepidopteran pests respectively;
- 59122, expressing the Cry34Ab1, Cry35Ab1 and PAT proteins, for resistance to lepidopteran pests and tolerance to glufosinate-ammonium herbicides.

The application was officially acknowledged by EFSA on 10 March 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.

[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).

The opinion of the EFSA Scientific Panel on GMOs was adopted on 28 June 2017 (EFSA Journal 2017;15(8):4921[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.
On 5 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-2013-118;
- The opinion of EFSA;
- The advices already adopted by the BAC on the single events and 11 subcombinations (stacked events). The conclusions of the BAC for the most recent applications for the single events were as follows:

Event	Application number	BAC advice	Conclusions
MON87427	EFSA-GMO-BE-2012-110	BAC/2015/0585 (08-09-2015)	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.
1507	EFSA-GMO-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)
MON88017	EFSA-GMO-CZ-2005-27	BAC/2009/1045 (13-07-2009)	No major risks for human and animal health or concerning the environment were identified.
59122	EFSA/GMO/RX-003	BAC/2017/0740 (19/09/2017)	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], as well as eleven combinations of two or more events.

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 1507 x MON88017 x 59122 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 five-stacked event, the Biosafety

[2] See https://www.efsa.europa.eu/en/efsajournal/pub/4921
[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.

Advisory Council agrees with the GMO panel of EFSA that the compositional data of GM maize MON87427 x MON89034 x 1507 x MON88017 x 59122, in comparison with its conventional counterpart, do not raise safety concerns.

3.2. Assessment of toxicity

The Biosafety Advisory Council has evaluated the safety of the newly expressed Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS 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 should not raise toxicological concerns.

3.3. Assessment of allergenicity

The Biosafety Advisory Council has evaluated the safety of the newly expressed Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS 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 MON87427 x MON89034 x 1507 x MON88017 x 59122-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 1507 x MON88017 x 59122 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 1507 x MON88017 x 59122 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, 1507, MON88017 and 59122, 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: Minority declaration
Annex II: 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_0591)

Minority declaration of P. Baret

Considering that:
(1) The stacked event MON87427 x MON89034 x 1507 x MON88017 x 59122 comprises one single event (1507) that was not properly tested for toxicity (test based on cohorts of 12 rats instead of the recommended sample of 64) and
(2) The substantial equivalence between the GM crop and the conventional counterpart is not proven as the thiamin content is significantly different between the GM crop and the conventional counterpart,
The lack of toxicity is far from being demonstrated and I will recommend a negative advice in absence of new elements on toxicity.

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O./ref.: WIV-ISP/41/BAC_2015_0591
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Compilation of comments of experts in charge of evaluating the application EFSA/GMO/BE/2013/118
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 (KATHO), 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/2013/118** concerns an application submitted by the company **Monsanto** for authorisation to place on the market genetically modified **MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 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
The applicant has not clearly shown why the stacked event MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 maize resulting in glyphosate & glufosinate-resistance is an advantage in comparison with the single event maize.

Single events dealing with CP4 EPSPS, PAT, Cry1A.105, Cry1F, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins have already been assessed and are 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 × 1507 × MON 88017 × 59122 maize is no reason to prohibit its import and processing in the EU.

A side effect of MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 maize may be an increased use of the herbicides glyphosate and glufosinate, and some health concerns about glyphosate and glufosinate have been reported. MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 is not intended for cultivation in the EU. Nevertheless, introduction of the MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 maize outside the EU may increase the use of glyphosate and glufosinate.

SBB Comment:
The assessment of the advantages of the newly expressed proteins or of use and safety of pesticides is not within the remits of the Biosafety Advisory Council.

Comment 2
No questions.

Comment 3
No comment.

Comment 4
The information provided in the application is sufficient.

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
No comment.

Comment 4
The information provided in the application is sufficient.

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, since MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 is a stacked event and produced through conventional breeding. It expresses the CP4EPSPS protein from MON 87427 and Cry1A.105, Cry2Ab2, Cry1F, Cry34Ab1, Cry35Ab1, CP4EPSPS and PAT-proteins from Mon89034x1507xMon88017x59122. These are assessed as being safe and without concerns for humans, farm animals and environment.
There is no evidence of any safety issues related to the use of the components of this stacked event for any of the donor organisms of the coding and non-coding DNA sequences present in MON 87427 and MON 89034 × 1507 × MON 88017 × 59122.

Comment 2
The dossier describes the stacking of different genes in maize using conventional breeding. Event MON 87427 has been approved for authorisation (EFSA-GMO-BE-2012-110). Event MON 89034 x 1507 x MON 89017 x 59122 has been approved for authorisation (EFSA-GMO-CZ-2008-62). 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 3
No comment.

Comment 4
The information provided in the application is sufficient.

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 87427 and MON 89034 × 1507 × MON 88017 × 59122 by conventional breeding results in a plant with full vegetative and male reproductive tolerance to glyphosate as well as tolerance to glufosinate-ammonium and additional insect resistance due to the expression of Cry-proteins as mentioned in A.2.1.

Comment 2
P 39: Table 4; MON 89034; expected fragment: it should be >4,1 Kb instead of <4,1 Kb; to be corrected

General comment on the bio-informatic analysis p 53-69

Although the methodology for analysing the flanking sequences is solid (searching several databases using Blast programs) and the separate events (eg MON 87427 and MON 89034 x 1507 x MON 89017 x 59122) 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).

See also comment dossier MON 87427 x MON 89034 x NK603.
Note SBB : This comment is "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 then in silico bio-informatic analyses."

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
No questions

Comment 2
MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 is further referred to as MON 118.
MON 118 is obtained by conventional breeding of five varieties, which have been found to be compositional equivalent to conventional maize. Substantial differences in composition are not to be expected.

MON 118 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
No questions

Comment 2
The information provided in the application is sufficient.

A.3.3. COMPOSITIONAL ANALYSIS

Comment 1
The results of the compositional analysis showed that 61 of the 63 components assessed were found to be equivalent to commercial conventional reference hybrids at a 95% confidence interval, with only vitamin B1 in grain categorized as equivalent more likely than not, and equivalence limits which could not be well established for ADF in forage, due to a lack of observable variation in the values for the commercial conventional reference hybrids. These results confirm the compositional equivalence of MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 to conventional maize hybrid varieties.

Comment 2
Nutrient levels in grain: when MON 87427 and MON 89034 × 1507 × MON 88017 × 59122 treated with glufosinate and glyphosate (T) or non-treated (NT) are compared with the near isogenic control parental line as well as with conventional reference hybrids, all traits measured (protein, amino-acids, fatty acids, fiber, minerals, vitamins except Vit B1, anti-nutrients, secondary metabolites) were equivalent (category I) with outcome type 1 (no difference with control at 10% level) or type 2 (difference at 10% level).
Only Vit B1 was judged non-equivalent more likely than not (category 4), with somewhat lower levels in the stacked event.
However, when evaluated relative to the range of individual replicate values for control and to the range of conventional reference hybrids, the difference are of no relevance from a food and feed perspective. Moreover, for the comparison of the stacked event (NT, non-treated) with control parental line and conventional reference hybrids, as for Vit B1 in grain, the result showed then an equivalence category II (more likely equivalent than not) in the evaluation.
I can agree therefor with the conclusion that the introduced traits in MON 87427 and MON 89034 × 1507 × MON 88017 × 59122 are not major contributors in compositional variability in maize, hence that there is compositional equivalence of the stacked event to the conventional maize hybrid varieties.

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
- amino acids, 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 the study.

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 118 treated with glyphosate,
- MON 118 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 Cry1A.105 protein in grain is significantly higher in this hybrid compared to the parent line MON 89034. Based on calculated MOEs this seems to be of no concern.

The amount of CP4 EPSPS protein in this hybrid is also higher than in MON 87427 and MON 88017 due to the presence of two copies of the *cp4 epsps* gene. This seems to be of no concern according to the calculated MOEs.

The amounts of Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1 and Cry35Ab1 are comparable between hybrid and parent plant. The amounts of PAT protein are below the limit of detection.

Furthermore, there seem to be no problems 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. 119, arthropod damage: it is stated that in the individual site assessment, no differences were observed between the stacked event and the conventional control for any of the 84 comparisons for the assessed arthropods. But what about insect resistance in the stacked event as conferred by the Cry-proteins (mentioned on p. 31)? Are these arthropods also included in these comparisons

mentioned here? If so, I should expect differences; if not, I should not expect differences, but then, don't you miss some meaningful information about the effectiveness of these Cry-proteins and cry-gene insertions?

Comment 2
In section 3.4.3 Environmental Interaction evaluations it is mentioned that no differences were observed between MON 118 and the conventional control for any of the 100 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.

Comment 2
The applicant states that, with the exception of the introduced traits, MON 118 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 × 1507 × MON 88017 × 59122 maize will pose serious risks for toxicity. Single events MON 87427 (EFSA, 2015) and MON 89034 × 1507 × MON 88017 × 59122 maize (EFSA, 2010) 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.
Notwithstanding the controversy with regard to the effect of stacked events on human and animal safety (Pilacinski et al., 2011; Agapito-Tenfen et al., 2014; Kok et al., 2014; Zdziarski et al., 2014), it is assumed that 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 CP4 EPSPS, PAT, Cry1A.105, Cry1F, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins in MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 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 animal exposure

Comment 3
Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, CP4 EPSPS and PAT proteins are shown to be rapidly digested in simulated gastric and/or intestinal fluids. This was demonstrated in earlier studies.
Several acute oral toxicity studies with Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, Cry34Ab1/Cry35Ab1, CP4 EPSPS or PAT proteins have demonstrated that these proteins are not acutely toxic and do not cause any adverse effects.
The results of these data indicate that no biologically relevant sequence similarities were observed between the Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, CP4 EPSPS and PAT proteins and toxins, or biologically active proteins.

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
No questions or comments. Not applicable

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

Comment 1
No questions or comments

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 or comments

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 × 1507 × MON 88017 × 59122 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 × 1507 × MON 88017 × 59122 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.142.

Comment 2
No questions or comments

Comment 3
This hybrid GMO maize cumulates a significant number of new genes introduced into the plant. Each of the individual genes and their products 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 databases of 2012 to 2013. Also these updated analyses failed to indicate an increased risk of

allergenicity for the individual proteins. Obviously, these analyses only provide an indication of and as a consequence hold a level of uncertainty.
While this uncertainty can be considered as acceptable when looking at a single trait, it increases exponentially when looking at multiple traits stacked in a single organism. Thus when looking at the individual traits, there are in my opinion no objective reasons to expect an increased risk. The increased statistic uncertainty that accompanies (>7) stacked events may however suggest the opposite. Two actions may help to lower this concern:
1. A sequence and structure comparison of the inserted proteins in order to verify to what extend the inserted proteins share certain sequences, and if so, to what extend these specific shared sequences show similarity with known allergens. Shared sequences might also mean that intake exposure is higher than otherwise with a single event, hence increasing the risk.
2. A well-controlled independent follow up study in an exposed population verifying whether the incidence of maize allergy increases.

I have otherwise no comments on this very complex dossier.

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

Comment 1
No questions or comments

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 or comments

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 × 1507 × MON 88017 × 59122 maize.

Comment 2
No questions or comments

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 × 1507 × MON 88017 × 59122 maize.
Appenzeller et al. (2009) tested 1507 x 59122 maize grain in rats and found that it is as safe and nutritious as non-GM maize grain. Events 1507 and 59122 are parts of MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 maize. 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

B. EXPOSURE ASSESSMENT - ANTICIPATED INTAKE/EXTENT OF USE

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

C. RISK CHARACTERISATION

Comment 1
No questions or comments.

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 or comments.

Comment 2
The information provided in the application is sufficient.

E. ENVIRONMENTAL RISK ASSESSMENT

E.1. INTRODUCTION

Comment 1
No questions or comments

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 × 1507 × MON 88017 × 59122 maize is not intended for cultivation in the EU. Nevertheless, it may have consequences in countries were cultivation of MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 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 same herbicides may result in an increased accumulation of residues in plant tissues (Bøhn et al., 2014; Rubio et al., 2014). Health concerns with regard to the use of glyphosate and glufosinate, and their 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; Laugeray 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. Herbicide mixing of glyphosate and glufosinate exposes weeds to multiple mechanisms of action, which may delay resistance evolution, at least temporarily. But using herbicide mixes may increase the quantity of herbicidal compounds required. Herbicide mixtures are not a permanent solution to the problem of herbicide resistance, as they do not prevent it on the long run (Evans et al., 2015).

SBB Comment:

The use and safety of pesticides is not within the remits of the Biosafety Advisory Council.

Comment 2

No questions or comments

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

Comment 2
Not relevant.

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

Comment 1
No questions or comments.

Comment 2
Not relevant.

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

Comment 1
No questions or comments.

Comment 2
Not relevant.

E.3.6. EFFECTS ON BIOGEOCHEMICAL PROCESSES

Comment 1
No questions or comments.

Comment 2
Not relevant.

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.

References

Ackermann, W., Coenen, M., Schrödl, W., Shehata, A.A., Krüger, M. 2015. The influence of glyphosate on the microbiota and production of botulinum neurotoxin during ruminal fermentation. Curr. Microbiol. 70, 374-382.

Agapito-Tenfen, S.Z., Vilperte, V., Benevenuto, R.F., Rover, C.M., Traavik, T.I., Nodari, R.O. 2014. Effect of stacking insecticidal cry and herbicide tolerance epsps transgenes on transgenic maize proteome. BMC Plant Biol. 14, Article 346 (18 pp).

Benbrook, C.M. 2012. Impacts of genetically engineered crops on pesticide use in the U.S. – the first sixteen years. Env. Sci. Eur. 24, Article 24 (13 pp).

Bøhn, T., Cuhra, M., Traavik, T., Sanden, M., Fagan, J., Primicerio, R. 2014. Compositional differences in soybeans on the market: Glyphosate accumulates in Roundup Ready GM soybeans. Food Chem. 153, 207-215.

Carman, J.A., Vlieger, H.R., Ver Steeg, L.J., Sneller, V.E., Robinson, G.W., Clinch-Jones, C.A., Haynes, J.I., Edwards, J.W. 2013. A long-term toxicology study on pigs fed a combined genetically modified (GM) soy and GM maize diet. J. Org. Syst. 8, 38-54.

Cheftel, J.C. 2005. Food and nutrition labelling in the European Union. Food Chem. 93, 531-550.

EFSA, 2010. Scientific Opinion on application (EFSA-GMO-CZ-2008-62) for the placing on the market of insect resistant and herbicide tolerant genetically modified maize MON 89034 x 1507 x MON 88017 x 59122 and all subcombinations of the individual events as present in its segregating progeny, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Dow AgroSciences and Monsanto. EFSA J. 8, 1781, 37 pp

EFSA, 2015. Scientific Opinion on the application (EFSA-GMO-BE-2012-110) for the placing on the market of tissue-selective herbicide-tolerant genetically modified maize MON 87427 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. EFSA J. 13, 4130, 25 pp

Evans, J.A., Tranel, P.J., Hager, A.G., Schutte, B., Wu, C., Chatham, L.A., S Davis, A. 2015. Managing the evolution of herbicide resistance. Pest Manag Sci. 71, in press, doi: 10.1002/ps.4009.

Fernandez-Cornejo, J., Wechsler, S., Livingston, M., Mitchell, L., 2014. Genetically Engineered Crops in the United States, Economic Research Report 162, USDA, Washington DC, USA. 54 pp.

Garry, V.F., Harkins, M.E., Erickson, L.L., Long-Simpson, L.K., Holland, S.E., Burroughs, B.L. 2002. Birth defects, season of conception, and sex of children born to pesticide applicators living in the Red River valley of Minnesota, USA. Environ. Health Perspect. 110, Suppl 3, 441-449.

Gasnier, C., Dumont, C., Benachour, N., Clair, E., Chagnon, M.C., Seralini, G.E. 2009. Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. Toxicology 262, 184-191.

George, J., Prasad, S., Mahmood, Z., Shukla, Y. 2010. Studies on glyphosate-induced carcinogenicity in mouse skin: A proteomic approach. J. Proteomics 73, 951-964.

Guilherme, S., Santos, M.A., Gaivão, I., Pacheco, M. 2014. DNA and chromosomal damage induced in fish (Anguilla anguilla L.) by aminomethylphosphonic acid (AMPA) - the major environmental breakdown product of glyphosate. Environ. Sci. Pollut. Res. 21, 8730-8739.

Guyton, K.Z., Loomis, D., Grosse, Y., El Ghissassi, F., Benbrahim-Tallaa, L., Guha, N., Scoccianti, C., Mattock, H., Straif, K. 2015. Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. Lancet Oncol. 16, 490-491.

Kok, E.J., Pedersen, J., Onori, R., Sowa, S., Schauzu, M., De Schrijver, A., Teeri, T.H. 2014. Plants with stacked genetically modified events: to assess or not to assess? Trends Biotechnol. 32, 70-73.

Krüger, M., Schledorn, P., Schrödl, W., Hoppe, H.W., Lutz, W., Shehata, A.A. 2014. Detection of glyphosate residues in animals and humans. J. Environ. Anal. Toxicol. 4, Article 210, 5 pp.

Laugeray, A., Herzine, A., Perche, O., Hébert, B., Aguillon-Naury, M., Richard, O., Menuet, A., Mazaud-Guittot, S., Lesné, L., Briault, S., Jegou, B., Pichon, J., Montécot-Dubourg, C., Mortaud, S. 2014. Pre- and postnatal exposure to low dose glufosinate ammonium induces autism-like phenotypes in mice. Front. Behav. Neurosci. 8, Article 390 (14 pp).

Mesnage, R., Defarge, N., Spiroux de Vendômois, J., Séralini, G.E. 2014. Major pesticides are more toxic to human cells than their declared active principles. BioMed Res. Int. Article ID 179691, 8 pp.

Pilacinski, W., Crawford, A., Downey, R., Harvey, B., Huber, S., Hunst, P., Lahman, L.K., MacIntosh, S., Pohl, M., Rickard, C., Tagliani, L., Weber, N. 2011. Plants with genetically modified events combined by conventional breeding: An assessment of the need for additional regulatory data. Food Chem. Tox. 49, 1-7.

Rubio, F., Guo, E., Kamp, L. 2014. Survey of glyphosate residues in honey, corn and soy products. J Environ. Anal. Toxicol. 4: 249. doi: 10.4172/2161- 0525.1000249, 8 pp.

Samsel, A., Seneff, S. 2013. Glyphosate's suppression of cytochrome P450 enzymes and amino acid biosynthesis by the gut microbiome: Pathways to modern diseases. Entropy 15, 1416-1463.

Seneff, S., Swanson, N., Li, C. 2015. Aluminum and glyphosate can synergistically induce pineal gland pathology: Connection to gut dysbiosis and neurological disease. Agric. Sci. 6, 42-70.

Steiner, H.Y., Halpin, C., Jez, J.M., Kough, J., Parrott, W., Underhill, L., Weber, N., Hannah, L.C. 2013. Evaluating the potential for adverse interactions within genetically engineered breeding stacks. Plant Physiol. 161, 1587-1594.

Zdziarski, I.M., Edwards, J.W., Carman, J.A., Haynes, J.I. 2014. GM crops and the rat digestive tract: A critical review. Environ. Int. 73, 423-433.

Zouaoui, K., Dulaurent, S., Gaulier, J.M., Moesch, C., Lachâtre, G. 2013. Determination of glyphosate and AMPA in blood and urine from humans: About 13 cases of acute intoxication. Forensic Sci. Int. 226, e20-e25.