Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on application EFSA-GMO-NL-2015-124 (maize MON 87411) from Monsanto under Regulation (EC) No. 1829/2003

11 September 2018
Ref. SC/1510/BAC/2018_0704

Context

Application EFSA-GMO-NL-2015-124 was submitted by Monsanto for the authorisation for the marketing of genetically modified (GM) maize MON 87411 for food and feed uses, import and processing (excluding cultivation) within the European Union, within the framework of Regulation (EC) No. 1829/2003[1].

Maize MON 87411 contains the DvSnf7 dsRNA expression cassette for down-regulation of the *Snf7* gene transcript in corn rootworm, leading to pest mortality after consumption, and contains genes that express the Cry3Bb1 and CP4 EPSPS proteins, conferring resistance to corn rootworms and tolerance to glyphosate herbicides respectively.

The application was validated by EFSA on 17 August 2015 and a formal three-month consultation period of the Member States was started, lasting until 26 November 2015, 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). 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 sent to EFSA on 19 November 2015.

The opinion of the EFSA Scientific Panel on GMOs was published on 28 June 2018 (EFSA Journal 2018;16(6):5310[2]) together with the responses from the EFSA GMO Panel to comments submitted by the Member States during the three-month consultation period.
On 11 July 2018 these two 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 information below:
- The comments formulated by the experts on application EFSA-GMO-NL-2015-124; and
- The opinion of EFSA.

[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.2018.5310

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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 87411 (i.e. during transport and/or processing) into the European environment[3] 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

The Biosafety Advisory Council agrees with the GMO panel of EFSA that the compositional data of GM maize MON 87411, in comparison with its conventional counterpart, do not raise safety concerns.

3.2. Assessment of toxicity

The Biosafety Advisory Council agrees with the GMO panel of EFSA that the available data on the toxicity of GM maize MON 87411, in comparison with its conventional counterpart, does not raise safety concerns.

3.3. Assessment of allergenicity

The Biosafety Advisory Council has evaluated the safety of the newly expressed CP4 EPSPS and Cry3Bb1 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 therefore agrees with the GMO panel of EFSA that the available data on the allergenicity of soybean MON 87751, in comparison with its conventional counterpart, does not raise safety concerns.

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 87411-derived food and feed are not expected to differ from those of conventional maize varieties.

4. Monitoring

With regard to monitoring, the Biosafety Advisory Council is of the opinion that the information provided is sufficient.

[3] 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.

Conclusion

Based on the whole set of data on maize MON 87411 provided by the applicant, the scientific assessment of the dossier done by the Belgian experts, the opinion of EFSA, and the answers of the EFSA GMO panel to the questions raised by the Belgian experts, the Biosafety Advisory Council:

1) Agrees with the GMO panel of EFSA that the potential environmental release of maize MON 87411 is unlikely to pose any threat to the European environment;
2) Agrees with the GMO panel of EFSA that in the context of its proposed uses, maize MON 87411 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 the existing allergenicity monitoring systems.

Dr. Corinne Vander Wauven
President of the Belgian Biosafety Advisory Council

Annex I: Compilation of comments of experts in charge of evaluating the application EFSA-GMO-NL-2015-124 (ref. BAC_2015_0773)

Bioveiligheidsraad
Conseil de Biosécurité

19/11/2015

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC_2015_0773
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Compilation of comments of experts in charge of evaluating the application EFSA/GMO/NL/2015/124
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 8 September 2015.
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, maize, breeding techniques, agronomy, plant biology.
SBB: Didier Breyer, Fanny Coppens, Martine Goossens, Katia Pauwels

- **INTRODUCTION**

Dossier **EFSA/GMO/NL/2015/124** concerns an application submitted by the company **Monsanto** for authorisation to place on the market genetically modified **Maize MON 87411** 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 19 August 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
Some concerns are formulated with regard to the safety of MON 87411 maize, containing Cry3Bb, CP4 EPSPS proteins and DvSnf7. Cry3Bb and CP4 EPSPS were considered as safe for human and animal health. However, the controversy with regard to the degradation of dsRNA in the digestive tract of humans and livestock warrants further investigation.

No direct effect of the genetic modification of MON 87411 maize, but an indirect effect may be an increased use of glyphosate. Some health concerns about glyphosate, have been reported. MON 87411 maize is not intended for cultivation in the EU. Nevertheless, introduction of MON 87411 maize elsewhere may increase the use of these herbicides. As a consequence, imported maize, destined for food and feed use, may contain residues of glyphosate or/and metabolites. Therefore, cultivation of MON 87411 maize should meet the restrictions specific to herbicide-treated crops. Monsanto should make efforts to guarantee that these restrictions are complied.

General comment with regard to the robustness of this dossier: referring to the incomplete data provided previously in other dossiers by other applicants (Windels et al., 2001; EFSA, 2015), the robustness of the evaluation of this dossier is based on the reliability and the integrity of the applicant in question. I do hope the applicant will respect his promise (P.212 of the technical dossier; 6.5. Reporting the results of monitoring) and inform the European Commission if information that confirms an adverse effect of MON 87411 maize and that alters the existing risk assessment becomes available.

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

Comment 2
No comments.

Comment 3
No comment, adequate information is provided.

Comment 4
No major comments, but on page 3 of the Summary text, the phrasing "…glyphosate. These proteins are identical…MON88017" is a bit misleading as it ignores the suppression cassette. A simple rephrasing will solve the problem.

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 major comments. But note the typo on page 9: Fusarium and not FU.S.rium.

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
Cry3Bb1-protein: expressed already in MON88017 and MON89034 x 1507 x MON88017 x 59122 (stacked event).

DvSnf7 suppression cassette:
- Discussion upon uptake of ingested plant miRNA's: apparently present in mice tissues.
- Despite consumption of miRNA's, horizontal delivery via oral ingestion (study in man, mice and honey bee, nonhuman primates) is neither frequent nor prevalent (study of Snow, 2013; Witwer et al., 2013).
Conclusion: RNA molecules mediating gene suppression in plants have history of safe use and does not pose any adverse effects to human or animal health.

No biological relevant sequence similarities between Cry3Bb1 and CP4EPSPS proteins and allergen, toxin or other biologically active proteins that could be harmful to human or animal health.

Comment 2
No comments.

Comment 3
No comment, adequate information was provided.

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
Very good diagram on p38 and 39, and explanation of the endosomal-autophagy pathway in degradation of receptors (or absence of it in DvSnf7-deficient cells of corn rootworm).

- Potential risk associated with horizontal gene transfer: negligible through physical, physiological and biochemical barriers and DNA degradation processes in man and animals.
- DvSnf7 suppression cassette, cry3Bb1 expression cassette and cp4epsps expression cassette were integrated in the maize genome at a single locus; the stability of the insert was demonstrated across multiple generations.

Comment 2
No comments.

Comment 3
Molecular characterization of MON 87411 maize was adequately carried out and the results do not raise any safety concern.

Comment 4
Comment #1-
NGS/JSA is used for the molecular characterization, i.e. for insert number determination, control of absence of unintended DNA sequences from plasmid vector, and for generational study of insert stability. However, the applicant provides limited information allowing the risk assessor to validate the method in the particular case of the application. In particular, Carleton et al 2012 say (CBI- report Carleton 2012 page 14, and main text page 43) : *The depth of coverage (the median number of times each base of the genome is independently sequenced) was ≥75x for each genome. It has previously been demonstrated that 75x coverage of the soybean genome is adequate to provide comprehensive coverage and ensure detection of inserted DNA (Kovalic et al., 2012) and similarly 75x coverage provides comprehensive coverage of the maize genome (Clarke and Carbon, 1976). The level of sensitivity of this method was demonstrated by detection of a positive control spiked at 1/10th copy-per-genome equivalent.*
It is unclear how the paper (Clarke and Carbon, 1976) was used to determine coverage depth of the maize genome. Positive controls are introduced but, in our view, the missing information is the variablity of coverage depth *across* the genome. Indeed, depending on eu-/heterochormatic regions, GC contents etc., variability is expected in sequencing efficacy and coverage depth along the genomic DNA and how this is taken into consideration is unclear to me. Considering that NGS/JSA methodology is rather new in the risk assessment of GMO, detailed justification of the assumptions made should be provided. This is needed for the quality check which is expected from risk assessors when analyzing the applicants' dossiers.

Comment #2 : In the section analysing putative tranlsation products of the ORFs within the insert, Page 9 of Kang and Silvanovich 2014b Part 1 (CBI) reads :
*"*confidential information* (Carleton et al., 2014)".* However, the reference Carleton et al 2014 (CI) is missing in the dossier and we could not analyse further this issue. The applicant is asked to provide this report. It has been published that the CaMVpromoter overlaps with protein-coding domains of the viral genome, which does not necessarily pose a risk (Podevin and du Jardin, 2012, GM Crops and Food, 3:296-300).

Comment #3 : In the analysis of putative translation products of the ORFs newly created by the genetic modification, when hits above fixed thresholds are found, the applicant has to conclude on the likelihood of expression, as the next step in the RA. In this dossier, two hits are found by the bioinformatics search of frames 5 and 6 translation products of the insert (FASTA algorithm and

PRT_2014 databases, see page 59 and 60 of Part II- main text), but no further analysis is provided. Instead, the applicant discards the two hits on the basis of the absence of 'biological relevance' but in the absence of convincing justification at that level (in our opinion), further analysis of the likelihood of expression should be provided, and this should be done by bioinformatics analysis before envisaging any experimental data at the RNA or polypeptide level.

Comment #4. When preparing protein samples for the ELISA analysis, no protease inhibitors were included in the extraction buffers (Beyene 2013a, CBI, pp.13-14). Although this option is likely explained by previous experience of the applicant with similar tissue samples and proteins, some justification should be given in the current dossier.

Comment 5
Minor comment on section 1.8 (g) of Summary text: better to state "no meaningful differences" instead of "no differences".

A.3. COMPARATIVE ASSESSMENT

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

Comment 1
Comparison with NL6169 which is the conventional counterpart of the GM MON87411, referring to figure 11, but there the LH244, LH287 and HCL645 are mentioned, but not NL6169 maize:
This is not very clear for me.

Coordinator comment:
According to figure 11, forage compositional analysis, protein analysis and RNA analysis was done with the R4F1 generation, which is a HCL645 × LH244 hybrid; NL6169 is also a LH244 × HCL645 hybrid (page 23) and thus a suitable comparator.
Analysis of the grain was with TI:BC1F1 generation, which is a HCL645 × LH244 hybrid, backcrossed to HCL645.

Comment 2
None.

Comment 3
Maize 87411 is compared with the appropriate conventional counterpart, grown at the same field sites during the same season. This is the traditional approach in this type of studies. The conventional counterpart has background genetics similar to Maize 87411 with the exception of the particular traits.

No particular remarks.

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

Comment 1
No questions.

Comment 2
No remarks.

A.3.3. COMPOSITIONAL ANALYSIS

Comment 1
Statistically significant differences between MON 87411 maize and the conventional counterpart were reported for several compounds. However, mean values are within the range of maize references, supporting the compositional equivalence between MON 87411 maize and conventional maize.

Comment 2
No questions; (see p78). Dimboa is not included in the anti-nutrients to be measured because of its too big variability and also trypsin- and chymotrypsin inhibitors are not included because to low levels in maize.

Comment 3
The OECD guidelines are followed for the selection of nutrients and anti-nutrients.

The nutrients and anti-nutrients selected were:
proximates: protein, total fat, moisture and ash,
carbohydrates by calculation
fiber: ADF,NDF and TDF
amino acids: relevant amino acids are included,
fatty acids: the analysis covers the whole range of important fatty acids
minerals: important minerals were analyzed
vitamins: folic acid, niacin, provit A, B1, B2, B6 and E
anti-nutrients: phytic acid and raffinose,
secondary metabolites: furfural, ferulic acid, p-coumaric acid.

As already mentioned before, the proposed fiber and carbohydrates approach is obsolete for human nutrition. Up to date methods for fiber analysis and for starch and sugars are nowadays widely applied.

SBB and coordinator comment:
EFSA has previously replied to this same question from our expert that "Analytical methodologies for specific endpoints are not defined in EFSA guidance documents". Consequently, this comment was not sent to EFSA.

No differences were observed for most constituents between maize 87411, treated and non-treated, and the conventional counterpart. However differences were observed for palmitic acid. The applicant states that the observed differences are of no relevance from a food and feed safety perspective.

The applicant concludes that maize 87411 is compositionally similar to conventional maize.
I agree with this conclusion.

Comment 4
Seems to be normal. No comments.

A.3.4. AGRONOMIC AND PHENOTYPIC CHARACTERISTICS

Comment 1
No questions; I can agree with the conclusion that MON87411 (T and NT) is compositionally similar to the conventional comparators and is not a significant contributor to compositional variability in maize.

Comment 2
None.

A.3.5. EFFECTS OF PROCESSING

Comment 1
No questions.

Comment 2
As maize 87411 is not different from conventional maize, no differences is processing technology are expected. The wet and dry milling processes are briefly reviewed.

No further questions.

A.4. TOXICOLOGICAL ASSESSMENT

A.4.1. METHODOLOGY USED FOR TOXICITY TESTS

Comment 1
Both proteins Cry3Bb1 and CP4EPSPS have a history of safe use, no structural similarity to known toxins or other biologically active proteins having adverse effects in humans or animals, and are rapidly digested in gastro-intestinal systems of mammals and birds, or “of vertebrates”. This would be better than only “mammalian gastrointestinal systems”, since maize is also fed to birds (and fish??).

As for DvSnf7-RNA, nucleic acids have a long history of safe consumption, and the hairpin secondary structure of dsRNA produced from DvSnf7 suppression cassette precludes translation initiation and protein synthesis; however, on p143, very low expression of DvSnf7 is envisaged; I guess this is not the protein, but RNA (cfr p 192)? There it is stated that DvSnf7 gene sequence do not produce any novel proteins. In the text it should made undoubtedly clear, and therefore the wording on p143 is confusing.

Coordinator comment:
On p. 143 reference is made to section 2.4.3.; section 2.4.3 repeatedly and clearly refers to DVsnf7 RNA and RNA only; the anticipated consumption of 0.003 µg/kg/day is unmistakably consumption of RNA according to section 2.4.3.

The MON87411-produced Cry3Bb1 and CP4EPSPS proteins are equivalent; therefore the existing protein safety data can be applied for MON87411-produced cry and EPSPS proteins:
Based on the evidence described on p142, there is no hypothesis to justify the use of experimental animals to conduct a 28-day toxicity study.

As for DvSnf7 RNA, no evidence for toxicity or allergenicity of ingested RNA in vertebrates, and proven with the 28-day repeat dose toxicity study in mice, and the 90-day toxicity study with corn grain from MON 87411.

Where are the data of this study? Under 1.4.4? Yes, but very little data given.

Coordinator comment:
MSL 0025235, 2013, provided in the CI

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

Cry3Bb1 and CP4 EPSPS in MON 88017 maize have been considered as safe for human and animal health (EFSA, 2011). However, there is some controversy with regard to the degradation of dsRNA's (DvSnf7) in the digestive tract of mammals. Humans and livestock commonly consume dsRNA from different dietary sources, without detrimental effects or adverse consequences (Witwer and Hirschi, K.D. 2014, Liu et al., 2015), because of the presence of nucleases in the gastrointestinal tract, which rapidly degrade ingested nucleic acids (Carver and Walker, 1995; WHO. 2000; Beever et al., 2003). Consequently, food and feed derived from crops utilizing RNA-based mechanisms are expected to be as safe as food and feed derived through conventional plant breeding (Petrick et al., 2013; 2015; Snow et al., 2013; Laubier et al., 2015). However, experiments showed absorption and proof of abundant plant nucleic acids in human and porcine breast milk exosomes (Spisák et al., 2013; Lukasik and Zielenkiewicz, 2014), or in blood and tissues of various animals (Zhang et al., 2012; Baier et al., 2014; Liang et al., 2015). Heinemann et al. (2013) recommended a proper assessment of the safety of dsRNA-producing GM organisms.

Coordinator comment:
The reports Zhang et al. 2012 and Heineman et al. 2013 are extensively discussed in the application (p.31-p.32). Similar arguments can be used with regard to more recent reports (Spisak et al. 2013; Lukasik and Zielenkiewicz, 2014; Baier et al. 2014; Liang et al. 2015) that are not discussed in the application.
In addition Lusk (2014) [Diverse and Widespread Contamination Evident in the Unmapped Depths of High Throughput Sequencing Data. PLoS ONE 9: e110808] argues that contamination can explain claims that nucleic acids pass from food to blood. The following sentence is appended to the above comment:
"The applicant is asked to comment on this discrepancy, taking into account Lusk (2014) [Diverse and Widespread Contamination Evident in the Unmapped Depths of High Throughput Sequencing Data. PLoS ONE 9: e110808]"

Comment 2

See under A.4.1.

Comment 3

Cry3Bb1 and EP4 EPSPS proteins: All requested studies and updated analysis were provided and confirmed that there should be no concerns regarding the potential toxicity of the Cry3Bb1 and EP4 EPSPS proteins. No further comments or questions.

Comment 4
Adequate assays and studies were carried out. The results do not raise any safety concern.

Comment 5
None.

Comment 6
The amounts of newly expressed proteins are similar to the levels in the parental lines.

CP4 EPSPS: Sequence homology with known toxins (Kang and Silvanovich, 2014c)

The results of these analyses indicate that there were no biologically relevant sequence similarities to toxins when the CP4 EPSPS protein sequence was used as a query for a FASTA search of the AD_2014 or TOX_2014 database. When searching the PRT_2014 database, results confirm that no biologically relevant structural similarity to proteins of concern was observed for CP4 EPSPS sequence.

Cry3Bb1: Sequence homology with known toxins (Kang and Silvanovich, 2014d)

The results of these analyses indicate that there were no biologically relevant sequence similarities to toxins when the Cry3Bb1 protein sequence was used as a query for a FASTA search of the AD_2014 or TOX_2014 database. When searching the PRT_2014 database, results confirm that no biologically relevant structural similarity to proteins of concern was observed for Cry3Bb1 sequence.

A.4.3. ASSESSMENT OF NEW CONSTITUENTS OTHER THAN PROTEINS

Comment 1
Not applicable.

Comment 2
DvSnf7 dsRNA:
Expression studies:
The DvSnf7 dsRNA levels in maize tissues (forage/grain) are higher in plants treated with glyphosate than in the tissues of untreated plants. Is there an explanation for this?
(Coordinator comment: The expression is actually lower in plants treated with glyphosate)
Potential off-target effects:
Direct feeding bioassays were performed which demonstrated that the RNAi effect observed is highly specific. Nevertheless, the molecular and functional equivalence between the *in vitro* (used in the assays) and plant-produced dsRNA was not demonstrated. Reference was made to scientific publications to show the history of safe exposure in human diets to dsRNA of various sizes and sequences. In addition, reference was made to a 28-day repeat toxicity study in mice with both siRNA and long dsRNA targeting the vATPase control gene.
Bioinformatics analysis was provided. No potential changes were identified that requires further characterisation.

Comment 3
Expression of a dsRNA in plant cells does not in itself raise a safety issue. Indeed, as RNA interference is a naturally occurring gene regulation mechanism in plants and animals, the presence of these interfering RNA in the diet does not raise specific safety concerns.

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

Comment 1
Not applicable.

Comment 2
No unintended compositional changes were detected in the compositional analyses (equivalence and difference tests). All statistically significant differences between the MON87411 and the NL6169 control key component levels were within the conventional control range values. No particular natural constituents of MON87411 maize are considered to be of significant concern to require additional information or further risk assessment. No further comments or questions.

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

Comment 1
No questions.

Comment 2
A 90-day rat feeding study is made available conducted according to the OECD TG408 guideline and the Scientific Opinion EFSA 2011c. No adverse effects were observed in this extensive study. The bodyweight of the male rats fed a diet containing 33% glyphosate-treated MON87411 was lower at week 11 and 12. However, this was not the case in female rats and no other treatment related adverse effects could be detected.
It was suggested by the author that the male control group had a slighter higher than normal mean body weight because the mean male control group values were higher than the study centre historical control data for pair-housed rats. Relative testes weight (g/100g body weight) was increased for the male rats fed a diet containing 33% glyphosate-treated MON87411, however no histopathological changes were observed in the testes.
The diet identity was confirmed by evaluating the presence of the Cry3Bb1 protein in the diet. It would have been useful to evaluate the presence of the target RNA.
In addition, as stated in the EFSA event report "International scientific workshop 'Risk assessment considerations for RNAi-based GM plants'" (EFSA supporting publication 2014:EN-705): A better understanding of the mechanisms, relevant for consumer exposure to RNAi molecules derived from RNAi-based plants, could be useful, including cross-kingdom transfer, uptake, and translocation in humans and animals. Are there already further improved detection methods for small RNA molecules in mammals available? The testing of the rat blood and the rat tissues for presence of the siRNAs should be done as the absence of the siRNAs can confirm the lack of off-target effects in mammals.
Further comment: No data is made available concerning the herbicide glyphosate and the metabolites residues in the MON87411 maize used for food/feed.
No further questions.

Comment 3
b) 90-Day rat feeding study.

Based on the results of this study, dietary administration of ground grain from MON 87411 for at least 90 consecutive days at a concentration of 33% (w/w) in the diet (equivalent to 1899 mg/kg of total cage body weight/day for males and 2303 mg/kg of total cage body weight/day for females) had no effects on the growth or health of Sprague Dawley (Crl:CD[SD]) rats.

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
It is assumed that MON 88017 maize does not pose a serious allergenic risk, and that it is comparable with conventional maize with regard to allergenicity.

Comment 2
No questions.

Comment 3
None.

Comment 4
MON 87411 contains three stacked events, namely the DvSnf7 suppression cassette, the *cry3Bb1* expression cassette, and the *cp4 epsps* expression cassette.

- Cry3Bb1 is also expressed in commercially available MON 88017 maize and MON 89034 × 1507 × MON 88017 × 59122 maize, both of which have been assessed by the EFSA as being as safe as their conventional counterpart and commercial reference varieties. Large scale cultivation of the Cry3Bb1-expressing crops did not provide an indication of a harmful health impact, thus further supporting the safety of the Cry3Bb1 protein regarding potential for eliciting allergic responses.
- Also the CP4 EPSPS protein has been extensively evaluated by EFSA in the context of previous applications for the placing on the EU market of different GM crops. Also here no (allergenicity) concerns were identified. Furthermore, the large scale cultivation of CP4 EPSPS-expressing crops similarly indicate that the CP4 EPSPS protein does not pose an increased risk for allergenicity.
- An updated bioinformatics analysis has been performed for both Cry3Bb1 and CP4 EPSPS looking for sequence similarities towards allergens using the AD_2014 allergen database obtained from 'Food Allergy Research and Resource Program Database' (FARRP, 2014). Also this updated analysis did not reveal an increased risk for allergenicity for both newly expressed proteins.
- The DvSnf7 suppression cassette in fact is made up of siRNA and therefore does not encode a protein or peptide. Digested RNA is rapidly broken down in single nucleotides and no cases of RNA acting as an allergen are known to my knowledge. I think it is therefore safe to conclude that the insertion of this suppression cassette does not pose a risk for allergenicity.

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

Comment 1
No questions.

Comment 2

Based on the above evidence assessment, it is quite safe to conclude that regarding both the *cry3Bb1* and the *cp4 epsps* expression cassettes, it is unlikely that these will affect the (lack of) allergenicity of the whole plant. A similar conclusion can be put forward for the *DvSnf7* suppression cassette. However, here off-target effects from the siRNA can never be excluded. Yet, the likelihood that such an off-target effect would act on a suppressor, hereby indirectly increasing the expression levels of some maize proteins that then would increase the allergenicity potential of the whole plant seems nevertheless to be very low to negligible. Maize not being an allergenic food, overexpression of an endogenous protein, which is not known to be allergenic, is unlikely to alter the overall allergenicity of the whole plant. I therefore agree with the applicant in stating that "MON 87411 and any food (or feed) derived from it, does not pose a significant allergenic risk to humans or animals".

A.5.3. ADJUVANTICITY

Comment 1
No questions.

Comment 2
No further comments.

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 88017 maize.

Comment 2
No questions.

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 88017 maize.

Comment 2
No questions.

B. EXPOSURE ASSESSMENT - ANTICIPATED INTAKE/EXTENT OF USE

Comment 1
No questions.

C. RISK CHARACTERISATION

Comment 1
No questions.

Comment 2
None (apart from the comments raised before in the MC section)

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

Comment 1
No questions.

E. ENVIRONMENTAL RISK ASSESSMENT

E.1. INTRODUCTION

Comment 1
No questions.

Comment 2
No comment, adequate information was provided.

E.2. GENERAL APPROACH OF THE ERA

Comment 1
No questions.

Comment 2
No comment, adequate information was provided.

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 comment, risk assessment was done adequately.

Comment 3

None

E.3.2. PLANT TO MICRO-ORGANISMS GENE TRANSFER

Comment 1
No questions.

Comment 2
No comment, risk assessment was done adequately.

Comment 3
None

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

Comment 1
Not applicable.

Comment 2
No comment, risk assessment was done adequately.

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

Comment 1
Not applicable.

Comment 2
No comment, risk assessment was done adequately.

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

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 88017 maize is not intended for cultivation in the EU. Nevertheless, an indirect effect of the approval of MON 88017 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 (Bøhn et al., 2014) and surface water (VMM, 2015). Health concerns with regard to the use of glyphosate have been reported (Ackermann et al., 2015; Guyton et al., 2015; Seneff et al., 2015). Food and feed that compromises human and animal health is unacceptable.

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

Comment 2
Not applicable.

Comment 3
No comment, risk assessment was done adequately.

E.3.6. EFFECTS ON BIOGEOCHEMICAL PROCESSES

Comment 1
Not applicable.

Comment 2
No comment, risk assessment was done adequately.

E.3.7. EFFECTS ON HUMAN AND ANIMAL HEALTH

Comment 1
Because RNA's were found in tissues of various mammals (see A.4.2), these aspects merit further investigation.

Comment 2
No questions

Comment 3
No comment, risk assessment was done adequately.

E.3.8. OVERALL RISK EVALUATION AND CONCLUSIONS

Comment 1
No questions.

Comment 2
No comment, risk assessment was done adequately.

Comment 3
None (apart from the comments raised before in the MC section)

E.4. POST MARKET ENVIRONMENTAL MONITORING PLAN

E.4.1. INTERPLAY BETWEEN ENVIRONMENTAL RISK ASSESSMENT AND MONITORING

Comment 1
No questions.

E.4.2. CASE-SPECIFIC GM PLANT MONITORING

Comment 1

No questions.

E.4.3. GENERAL SURVEILLANCE FOR UNANTICIPATED ADVERSE EFFECTS

Comment 1
No questions.

E.4.4. REPORTING THE RESULTS OF MONITORING

Comment 1
No questions.

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.

Baier, S.R., Nguyen, C.,Xie, F., Wood, J.R., Zempleni, J. 2014. MicroRNAs are absorbed in biologically meaningful amounts from nutritionally relevant doses of cow milk and affect gene expression in peripheral blood mononuclear cells, HEK-293 kidney cell cultures, and mouse livers. J. Nutr. 144, 1495-1500.

Beever, D.E., Glenn, K., Phipps, R.H. 2003. A safety evaluation of genetically modified feedstuffs for livestock production; the fate of transgenic DNA and proteins. Asian-Aust. J. Anim. Sci. 16, 764-772.

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.

Carver, J., Walker, W.A. 1995. The role of nucleotides in human nutrition. J. Nutr. Biochem. 6, 58-72.

EFSA, 2011. Scientific Opinion on application (EFSA-MO-CZ-2008-54) for placing on the market of genetically modified insect resistant and herbicide tolerant maize MON 88017 for cultivation under Regulation (EC) No 1829/2003 from Monsanto. EFSA J.; 9, 2428, 152 pp.

EFSA, 2015. Statement on the risk assessment of new sequencing data on GM maize event MIR604. EFSA J. 13, 4255, 6 pp.

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.

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.

Heinemann, J.A., Agapito-Tenfen, S.Z., Carman, J.A. 2013. A comparative evaluation of the regulation of GM crops or products containing dsRNA and suggested improvements to risk assessments. Environ. Int. 55, 43-55.

Laubier, J., Castille, J., Le Guillou, S., Le Provost, F. 2015. No effect of an elevated miR-30b level in mouse milk on its level in pup tissues, RNA Biol. 12, 26-29.

Liang, H., Zhang, S., Fu, Z., Wang, Y., Wang, N., Liu, Y., Zhao, C., Wu, J., Hu, Y., Zhang, J., Chen, X., Zen, K., Zhang, C.Y. 2015. Effective detection and quantification of dietetically absorbed plant microRNAs in human plasma. J. Nutr. Biochem. 26, 505-512.

Liu, Y., Zhang, Y., Dong, P., An, R., Xue, C., Ge, Y., Wei, L., Liang, X. 2015. Digestion of nucleic acids starts in the stomach. Sci. Rep. 5, Art. No. 11936, 11 pp.

Lukasik, A., Zielenkiewicz, P. 2014. In silico identification of plant miRNAs in mammalian breast milk exosomes – A small step forward? PLoS ONE 9, e99963, 10 pp.

Petrick, J.S., Brower-Toland, B., Jackson, A.L., Kier, L.D. 2013. Safety assessment of food and feed from biotechnology-derived crops employing RNA-mediated gene regulation to achieve desired traits: A scientific review. Regul. Toxicol. Pharm. 66, 167-176.

Petrick, J.S., Moore, W.M., Heydens, W.F., Koch, M.S., Sherman, J.H., Lemke, S.L. 2015. A 28-day oral toxicity evaluation of small interfering RNAs and a long double-stranded RNA targeting vacuolar ATPase in mice. Regul. Toxicol. Pharm. 71, 8-23.

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.

Snow, J.W., Hale, A.E., Isaacs, S.K., Baggish, A.L., Chan, S.Y. 2013. Ineffective delivery of diet-derived microRNAs to recipient animal organisms. RNA Biol. 10, 1107-1116.

Spisák, S., Solymosi N., Ittzés P., Bodor A., Kondor D., Vattay, G., Barták, B.K., Sipos, F., Galamb, O., Tulassay, Z., Szállási, Z., Rasmussen, S., Sicheritz-Ponten, T., Brunak, S., Molnár, B., Csabai, I. 2013. Complete genes may pass from food to human blood. PLoS ONE 8, e69805, 11 pp.

VMM. 2015. Pesticiden in oppervlaktewater en RWZI's in 2014. Vlaamse Milieumaatschappij. Aalst, 30 pp.

WHO. 2000. Safety aspects of genetically modified foods of plant origin. Report of a joint FAO/WHO expert consultation on f oods derived from biotechnology. World Health Organization, Geneva, Switzerland, 35 pp.

Windels, P., Taverniers, I., Depicker, A., Van Bockstaele, E., De Loose, M. 2001. Characterisation of the Roundup Ready soybean insert. Eur. Food Res. Technol. 213, 107-112.

Witwer, K.W., Hirschi, K.D. 2014. Transfer and functional consequences of dietary microRNAs in vertebrates: concepts in search of corroboration. Bioessays 36, 394–406.

Zhang, L., Hou, D., Chen, X., Li, D., Zhu, L., Zhang, Y., Li, J., Bian, Z., Liang, X., Cai, X., Yin, Y., Wang, C., Zhang, T., Zhu, D., Zhang, D., Xu, J., Chen, Q., Ba, Y., Liu, J., Wang, Q., Chen, J., Wang, J.,Wang, M., Zhang, Q., Zhang, J., Zen, K., Zhang, C.Y. 2012. Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA. Cell Res. 22, 107-126.