Bioveiligheidsraad
Conseil de Biosécurité

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Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2010_0371

Title: Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/NL/2007/39 from Monsanto under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/NL/2007/39 was submitted by Monsanto on 12 February 2007 for the marketing (import and processing) of the insect resistant and glyphosate-tolerant genetically modified MON89034 x MON88017 maize for food and feed uses under Regulation (EC) No. 1829/2003[1].

The application was officially acknowledged by EFSA on 20 September 2007. On the same date 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 (GMOs) being part of the products).

Within the framework of this consultation, the Belgian Biosafety Advisory Council, 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 Biosafety Advisory Council and the Division of Biosafety and Biotechnology (SBB). Eight experts answered positively to this request, and formulated a number of comments to the dossier, which were edited by the coordinator. See Annex I for an overview of all the comments and for the list of comments actually placed on the EFSAnet on 19 December 2007.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 10 March 2010 (The EFSA Journal, 2010, 8 (3):1564)[2], and published together with the responses from the EFSA GMO Panel to comments submitted by the experts during the three-month consultation period.

On 31 March 2010 the opinion of EFSA was forwarded to the Belgian experts. They were invited to give comments and to react if needed to the answers given by the EFSA GMO Panel, in particular in case the comments formulated in their initial assessment of the dossier were not taken into account in the opinion of EFSA.

The comments formulated by the experts together with the opinion of EFSA including the answers of the EFSA GMO Panel form the basis of the advice of the Biosafety Advisory Council given below.

[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: http://www.efsa.europa.eu/en/scdocs/scdoc/1564.htm

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In addition, the scientific evaluations of the single events, namely maize line MON89034 (EFSA/GMO/NL/2007/37) and maize line MON88017 (EFSA/GMO/CZ/2005/27), are taken into account in this advice. The Biosafety Advisory Council formulated a positive advice for each single event[3].

Scientific evaluation

1. Environmental risk assessment

According to the Biosafety Advisory Council no major risks were identified concerning the environment[4].

2. Molecular characterisation

With regard to the molecular characterisation, on request from the GMO panel of EFSA the applicant submitted complementary information. It was not reviewed by the Belgian experts but the Biosafety Advisory Council relies on the evaluation done by EFSA which 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
With regard to compositional analysis, the Biosafety Advisory Council is of the opinion that the information provided is sufficient and does not raise safety concerns.

3.2. Assessment of toxicity
With regard to toxicity, the Biosafety Advisory Council is of the opinion that the information provided is sufficient and does not raise safety concerns.

3.3. Assessment of allergenicity
Maize is not a major allergen source. The potential allergenicity of the newly introduced proteins has been assessed. With regard to allergenicity, the Biosafety Advisory Council is of the opinion that the information provided is sufficient and does not raise safety concerns.

3.4. Nutritional value
The Biosafety Advisory Council is of the opinion that the information provided is sufficient and shows the nutritional equivalence of the GM maize with its non-GM counterpart and conventional maize varieties.

4. Monitoring

As the allergenicity of the whole GM maize has not been assessed, it is recommended to take up monitoring of allergenicity as part of the general surveillance.

[3] Advice of BAC on maize line MON89034: BAC_2009_880; Advice of BAC on maize line MON88017: BAC_2009_01045
[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.

Conclusion

Based on the scientific assessment of the dossier done by the Belgian experts, taking into account the opinion of EFSA, the answers of the EFSA GMO Panel to the questions raised by the Belgian experts, the answers of the applicant to the EFSA GMO Panel questions and considering the data presently available, the Biosafety Advisory Council,

Agrees with the GMO panel of EFSA that
a) No major risks concerning the environment were identified.
b) No major risks for human and animal health were identified.

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

p.o.

Prof. D. Reheul
President of the Belgian Biosafety Advisory Council

Annex:
- Full comments of experts in charge of evaluating application EFSA/GMO/NL/2007/39 *and Comments submitted on the EFSAnet (ref. BAC_2007_PT_623)*

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Secretariaat
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Compilation of comments of experts in charge of evaluating the application EFSA/GMO/NL/2007/39
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 5 October 2007
Coordinator: Prof. dr. ir. Dirk Reheul
Experts: Pascal Cadot (Consultant), François Chaumont (UCL), Jacques Dommes (ULg), Patrick du Jardin (FUSAGx), André Huyghebaert (UGent), Jean-Pierre Maelfait (UGent), Peter Smet (Consultant), Wim Stevens (UIA), Bart Van Droogenbroeck (ILVO)
Domains of expertise of experts involved:
Improvement of plants, breeding, genetics, genome analysis, epigenetics, genetic engineering, molecular characterisation, agonomy, insect resistance, herbicide tolerance, transgene integration pattern, transgene expression, biochemistry, analysis of food/feed, industrial processing, toxicology, immunology, alimentary allergology, ecology, plant-insect relations, nature conservation, biosafety research, GMO traceability, monitoring, risk analysis.
Secretariat: Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

INTRODUCTION

Dossier **EFSA/GMO/NL/2007/39** concerns an application of the company **Monsanto** for the marketing of the genetically modified **maize MON89034 x MON88017** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 24 August 2007.
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. Comments placed on the EFSAnet are indicated in grey.

List of comments received from the experts

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1

The information is regarded as sufficient by the expert.

Comment 2

The notification concerns the authorization of MON 89034 x MON 88017 maize for import, processing, and food and feed use and not for cultivation.

B. INFORMATION RELATING TO THE RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANTS

Comments/Questions of the expert(s)

Comment 1

The information is regarded as sufficient by the expert.

Comment 2

The recipient plant is maize (*Zea mays* L.) that has been widely and extensively cultivated worldwide.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Comment 1

General comment : Both parental lines used for producing the hybrid subjected to this risk assessment are being evaluated as single events according to EC Regulation N° 1829/2003, as EFSA Application 37 for MON 89034 and EFSA application 27 for MON 88017. In accordance with the *EFSA guidance document of the Scientific Panel* on *Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events* (EFSA Journal (2007) 512, 1-5), where single events are evaluated under the same regulation and with the same scope of application, assessment of the stacked events is focused on the additional risks potentially associated with interactions and on the stability and the expression of the traits and proteins, that could impact their interaction with humans and with the environment. As a consequence, and considering that the full technical dossiers for the parental lines have not been provided to the expert, no opinion is

provided on the compliance to regulatory provisions of the parental events, nor on their safety. However, the expert is willing to do this extra-job in case of request of the Belgian competent authority.

Nevertheless, the **following conclusions** can be drawn on the hybrid dossier :

1. Integrity of the inserted sequences and integration sites with respect to the parental lines :

Although the probes used do not cover the entire length of the insert, the Southern blot analysis with chosen restriction enzyme-probe combinations are sufficient to conclude on the integrity and stability of the inserted DNA and of the flanking regions, during the conventional breeding of the hybrid.

2. Potential DNA interactions between the two inserts from the parental lines :

Although the two inserts from the two parental events share homologous sequences corresponding to promoter, leader, intron and transcription termination sequences, the expert is of the opinion that no increased instability of the inserts is expected in the hybrid, as compared with the single events. This point is further commented under section D.5.

Note from the SBB

Timing and availability explains why it is not always possible to have the same experts evaluating the parental lines and the hybrids. In case of this dossier, 3 of the experts have also evaluated both parental lines (Dommes, Smet, Stevens), 3 other experts (Cadot, Chaumont, Maelfait) have evaluated application 37 for MON 89034.

Comment 2

- **Table 2, pg 23 of the Technical Dossier, Part I**

When one verifies the genetic elements inherited from MON 89034 in the hybrid MON89034 x MON88017, with the genetic elements present in the original T-DNA (T-DNA I) on the plasmid that was used to generate MON 89034, two differences are detected. Length and name of right border sequences and of the P-e35S promoter have changed. This is explained on pg. 65-66 of the technical dossier EFSA-GMO-NL-2007-37 for event MON 89034. It would be useful to include the description of the recombination process that explains this 5' modification of the inserted T-DNA region in MON 89034 as relevant information in the dossier of the stacked event. In addition, in the summary (part II, pg. 12, Table 1) also the second left border has been typed with a superscript R2 to indicate recombination, though this is not the case. Literature reference for this left border is not given.

- **Figure 2, pg 25 of the Technical Dossier, Part I**

To avoid confusion, the same nomenclature as given in Table 2, pg 23 should be used to identify the elements in Figure 2: B-left border^{r1} and P-e35S^{89} instead of B-left border and P-e35S respectively. Same remark for Fig. 3, pg 26: CS-*cry3Bb1* is indicated in the figure, while CS-*MON 88017 cry3Bb1* is given in the table on pg 24.

Comment 3

MON 89034 x MON 88017 was produced by crossing inbred plants of MON 89034 and MON 88017 using traditional breeding. The maize plants contain the genetic modifications already present in the parents. No new specific genetic modification has been introduced.

D. INFORMATION RELATING TO THE GM PLANT

D.1 DESCRIPTION OF THE TRAITS AND CHARACTERISTICS WHICH HAVE BEEN INTRODUCED OR MODIFIED

Comments/Questions of the expert(s)

Comment 1

To be completed by the assessment of the parental inbred lines (single events).

Comment 2

All the necessary information is provided.

Comment 3

MON 89034 x MON 88017 produces three insecticidal proteins (Cry1A.105, Cry2Ab2 and Cry3Bb1) that protect the plants against different lepidopteran and coleopteran insect pests. It expresses also the CP4 EPSPS protein conferring tolerance to the herbicide glyphosate.

D.2. INFORMATION ON THE SEQUENCES ACTUALLY INSERTED OR DELETED

Comments/Questions of the expert(s)

Comment 1

Conformity of the sequences in the stacked event with those in the single events is established by appropriate Southern blot analysis, as commented under part C of this form.
However, the risk assessment of this hybrid must be completed by the thorough analysis of the parental inbred lines (single events), *e.g.* for the actual sequences of the inserts and flanking sequences (analysis of possibly interrupted genes, novel ORFs at the junctions sites etc.).

Comment 2

1. Same remark as for dossier EFSA/GMO/NL/2007/37 : for event MON 89034, the applicant did not describe the strategy and methods used to determine the sequence of plant genomic DNA flanking the insert.
2. The EFSA guidance document for the risk assessment of genetically modified plants (GMP) containing stacked events specifies that the intactness of flanking genomic DNA has to be checked. This was not done here, although it can be easily carried out by PCR. Nevertheless there is no scientific basis to support the fact that these sequences would be more unstable than any other region of plant genomic DNA.

Comment 3

- **Pg 30 and 31 of the Technical Dossier, Part I**

As there is no a priori or biological reason to assume that traditional interbreeding of independent GM lines will pose any additional risk through a compromised stability of copy number and insert structure, the applicant did not repeat laboratory analyses of copy number and insert integrity for MON 89034 x MON 88017. Southern blot analyses do demonstrate the identical organisation of the inserts and junction regions as characterised for the two parental events. However, these analyses do not allow to detect the small rearrangements within the detected fragments. But given the low likelihood of such processes to occur and the correct size of the expected fragments, this can be excluded.

Comment 4

Because it is considered that there is a low likelihood of molecular interactions between the inserts, the applicants did not start again a complete molecular analysis to demonstrate the size, copy number and integrity of the 2 inserts. Only two Southern blot analyses were performed and showed that the size of the inserts and flanking regions correspond to those of their respective parents. The size of the bands obtained in the control lanes including plasmid DNA cannot be understood from the technical dossier itself, but a detailed description of the Southern blot experiments is found in Groat et al. (2006).
It is also mentioned that both inserts are on separate chromosomes in the nuclear genome. A precise reference for this information should be given.

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1

The information is regarded as sufficient : appropriate control lines (non transformed maize and single events) and representative locations of cultivation allow to conclude that expression of the proteins of interest are in the range of those observed for the single events, hence raising no additional safety concern.

Comment 2

- **Pgs. 35 and 37 of the Technical Dossier, Part I**

Despite the fact that expression of *cry1A.105* and *cry3Bb1* are controlled by the same regulatory sequences (promoter, leader sequence and terminator) and have the same intron, no silencing effect can be observed, at least not reflected in the protein accumulation data collected by ELISA in the MON 89034 x MON 88017 event..
It is however not clear that the material characterised at the DNA level by Southern blot and that used to assess the protein accumulation levels by ELISA is the same. Can the applicant clearly describe what the source is of the material used for both assays?

Comment 3

The expression of the Cry and CP4 EPSPS proteins was assessed using an enzyme-linked immunosorbent assay (ELISA) in various plant tissues of MON 89034 x MON 88017 and the parents produced in 2005 in USA. The ranges of protein expression are comparable in the different maize lines.

D.4. INFORMATION ON HOW THE GM PLANT DIFFERS FROM THE RECIPIENT PLANT IN: REPRODUCTION, DISSEMINATION, SURVIVABILITY

Comments/Questions of the expert(s)

Comment 1

Field trials over several locations allow to conclude that agronomic and phenotypic characteristics of the hybrid is within the range of maize references, although some statistically significant differences have been shown when comparing the hybrid with the non transgenic maize line having the closest genetic background. This raises no safety concern, from an ecological viewpoint (*e.g.* invasiveness) or agricultural viewpoint (*e.g.* weediness).

Comment 2

I would find it valuable if the applicant could briefly describe, e.g. in a Table, the comparison of the climatic conditions at the field trial site location and the region where the product is intended to be used, in order to confirm the value of the results collected at the field trial site.

Comment 3

Some minor differences were observed for some phenotypic and agronomic characteristics between MON 89034 x MON 88017 and the control maize but seemed to be in the range of responses expected for maize.

D5. GENETIC STABILITY OF THE INSERT AND PHENOTYPIC STABILITY OF THE GM PLANT

Comments/Questions of the expert(s)

Comment 1

Although the applicant thoroughly discusses the likelihood of genetic instability due to homologous recombination in the hybrid, concluding in a convincing way that *no increased hazard or exposure is to be expected from potential recombination* (page 55 of the technical dossier), no attention is paid on potential interactions between the two T-DNA at the expression level. Indeed, two transcribed elements are shared by the two T-DNAs : the rice actin 1 intron (I-Ract1) and the wheat L-Cab leader sequences. **The applicant is invited to comment on possible co-silencing effects in this context**. This is in line with the EFSA guidance document on stacked events (EFSA Journal (2007) 512, 1-5),

which states, as regards possible interactions : *At the DNA level, this would include, for example, assessing possibilities for gene silencing.*

Besides this theoretical reasoning, empirical evidence is provided that expression of the traits of interest is maintained across the breeding history of the hybrid. No observation supports that the stacked traits could be less stable than the separate traits, **hence assessment of trait stability in the parental events seems sufficient. However, detailed analysis on this is not provided** in the stack dossier, hence attention should be given to the conclusions of the dossiers on the single events. In particular, the number of generations (seasons of trials or commercial releases) should be mentioned, as these data are lacking in the hybrid dossier.

Comment 2

All the necessary information is provided. No specific comments or suggestions.

Comment 3

The genetic stability of the insert was not tested. The applicants justified this by theoretical arguments based on previous studies on recombinations and concluded that it is appropriate to apply results of the characterisation performed on the parental lines MON 89034 and MON 88017. Demonstration of genetic stability of the inserts in marketed grains and in subsequent generations would be useful and fit with the guidelines for the safety assessment of genetically modified crops for food and feed use.

D.6. ANY CHANGE TO THE ABILITY OF THE GM PLANT TO TRANSFERR GENETIC MATERIAL TO OTHER ORGANISMS

Comments/Questions of the expert(s)

Comment 1

Cultivation is out of the scope of this application. In case of accidental release in the environment, no changed ability of the hybrid to transfer genes and traits to recipient organisms (plants, bacteria), as compared with the parental lines and with non GM maize varieties, is expected. No specific risk to the environment is identified.

D.7. INFORMATION ON ANY TOXIC, ALLERGENIC OR OTHER HARMFUL EFFECTS ON HUMAN OR ANIMAL HEALTH ARISING FROM THE GM FOOD/FEED

D.7.1 Comparative assessment

Comments/Questions of the expert(s)

Comment 1

In my comments maize Mon89034 x 88017 is further described as “proposed maize”.

The comparative assessment is based upon compositional analyses of the proposed maize and conventional maize with similar genetic background and other commercially available maize hybrids. The samples originate from field trials in the USA at five different locations.
Analyses of forage and grain were performed according to the OECD document. These include proximate analysis and a broad range of vitamins, minerals, anti-nutrients and secondary plant metabolites.
Comment: relevant constituents are covered including vitamins; I refer among others to sensitive vitamins like folic acid, and niacin typical for maize.
I will not repeat my comments on dietary fibre[1] as I understood that an actualization of the OECD document is highly needed for some nutrients important in human nutrition.

I agree with the conclusion that the proposed maize is compositionally equivalent to conventional maize and forage.

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

No remarks on locations, growing seasons, geographical spread and number of replicates.

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

Constituents for analysis are selected according to the OECD documents.
Relevant nutrients are well covered, as mentioned above. My point of view on dietary fibre is known (see footnote below).

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

No further comment.

[1] Comments already transmitted to EFSA for previous dossiers and repeated for this dossier:
Information on fibre is limited to acid detergent fibre and neutral detergent fibre. This is one of the approaches for animal feed. It is however not appropriate for human nutrition where concepts as dietary fibre, soluble fibre and insoluble fibre are widely used. A more in depth study of fibre composition is even relevant as different fibre constituents may have different functionality in human nutrition.

D.7.5 Product specification

Comments/Questions of the expert(s)

Comment 1

No further comment.

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1

Taking into account the compositional equivalence of the proposed maize with conventional maize it is not expected that any significant effect on processing will be detected.
The main applications of maize for feed, food and industrial (ethanol) uses are reviewed. These include the wet and dry milling processes. Intermediate and final products are discussed.
I agree with the conclusion that it is not expected that product obtained from the proposed maize will be different from the conventional products.

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

Economical aspects such as supply, demand and use of maize in the E.U. are discussed.
I have no further comment on the conclusion that there are no anticipated changes in the intake and/or extend of use of maize or products as a result of the addition of the proposed maize.

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

MON 89034 x MON 88017 is obtained from traditional breeding using MON 89034 and MON 88017. So no new genetic modifications were introduced.

Cry1A.105 and Cry2Ab2

Insecticidal proteins which impart protection against feeding damage caused by the European corn borer and other lepidopteran insect pests.

Cry3Bb1

Bacterial protein providing protection against certain coleopteran insect pests.

CP4 EPSPS

Provides tolerance against glyphosate herbicide.

Comment 2

No comment

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

Cry1A.105, Cry2Ab2 (both MON 89034) and Cry3Bb1 and CP4 EPSPS (MON 88017) proteins were tested in earlier studies. These studies showed no evidence of acute toxicity. Further testing of these proteins for acute toxicity is not required.

Based on the accumulated knowledge of Bt Cry proteins, a generalized mode of action has been proposed and includes the following steps: ingestion of crystals by the insect, solubilization of the crystals in the insect midgut, proteolytic processing of the released Cry protein by digestive enzymes to activate the toxin, binding of the toxin to receptors on
the surface of midgut epithelial cells of target organisms, formation of membrane ion channels or pores, and consequent disruption of cellular homeostasis (English, 1992). Electrolyte imbalance and pH changes render the gut paralyzed, which causes the insect to stop eating and die (Sacchi et al., 1986).

Is there scientific evidence available which indicates the total absence of such type of receptors in organisms, other than the above mentioned target organisms? If such evidence is not available, it may be appropriate to look for long-term effects of these proteins in organisms which could possibly come into contact with the Cry proteins.

Comment 2

No comment.

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Comment 1

See above, no comment.

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1

No further comment, see above.

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

The ranges across all sites for the Cry1A.105, Cry2Ab2, Cry3Bb1 and CP4 EPSPS protein levels in MON 89034 × MON 88017 were comparable to the corresponding ranges in either MON 89034 or MON 88017 (See tables 4, 5, 6 and 7 of the technical dossier).

Comparison of broiler performance and carcass parameters when fed diets containing MON 89034 x MON 88017, control or commercial corn (Davis, 2006).

There were no biologically relevant differences in the parameters measured between broilers fed the MON 89034 x MON 88017 diet and the control diet. In addition, based on individual treatment comparisons, broilers in general performed and had similar carcass yields and meat composition regardless of whether the diets contained MON 89034 x MON 88017, the control, or conventional reference corn.

13-Week feeding study in rats.

This study **should be performed** since synergistic effects of the proteins under investigation cannot be excluded beforehand.

Comment 2

Results of a feeding study of broiler chickens is included. No further comments.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

MON 89034 and MON 88017 have already been evaluated for their allergenicity:

Maize itself (Zea mais) rarely induces allergic reactions in man as a food nor as a pollination plant (heavy pollen)

MON 89034:
To study the allergenicity of the Cry1A.105 and Cry2Ab2 proteins Monsanto has used the following criteria to test for allergenicity:

1. the protein is from a non-allergenic source: hithereto there are no reports on allergenic properties of Bt proteins.
2. the protein does not share structural similarities to known allergen based on the amino acid sequence: no relevant matches were found using the AD6 database for both proteins ore aminoacid sequences. There is no significant similarity between Cry1A.105 and a kiwi fruit protein.. There were no alignments of at least 8 aminoacids found for Cry1A.105.
3. the protein is rapidly digested in simulated gastric fluid (SGF):
4. the protein respresents only a very small portion of the total protein in the grain

Nevertheless these rules are not absolute:

- a protein or polypeptide inserted in an other protein can and up with conformational changes of the original protein. Allergens are non only linear epitopes but can be formed by conformational epitopes.
- The rapid digestibility of a protein does not warrant non-allergenicity; some labile proteins are allergenic (eg. Mal d 1 form apple)
- The quantity of the protein in food is not absolutely related to allergenicity: allergic reactions can be induced by minute amounts of allergen

Post marketing surveillance remains necessary.

MON 88017

The new proteins Cry3Bb1 and CP4 EPSPS were already evaluated for allergenicity in the context of MON 863 and NK603 maize.
The risk for allergenicity can be assessed by combining different approaches (Helm 2003):

- content of the protein(s) in the food/feed
- digestibility of the protein(s) and stability in acid proteases in the food/feed
- comparison of the amino acid structure of the protein(s) with known allergens
- testing with specific IgE from allergic patients
- testing in animal models

For three of these parameters the proteins Cry3Bb1 and CP4 EPSPS showed a good profile:

- low content of proteins Cry3Bb1 and CP4 EPSPS in the maize end product
- good digestibility in acid peptic digestion

It has to be mentioned nevertheless that not all allergens are stable proteins (eg Mal d 1 from apple) (Ebo et al. 2005)

As far as the comparison of the proteins Cry3Bb1 and CP4 EPSPS with known allergen structures is concerned:

protein Cry3Bb1 showed some similarity with the Anisakis simplex tropomyosin Ani s3. The overlap of 120 aa contained four gaps and showed 27.5 % identity with an E score of 1.1. The longest stretch of continuous aa was 3; this was considered as non significant. Follow up of this situation is advised

since tropomyosin are to be considered as pan-allergen in a high number of living animal, with possible cross reactivity (Ebo and Stevens 2001).

protein CP4 EPSPS had an alignment of 30.5 % identity with Dermatophagoides farinae Der f 2over 82 aa with a high E score of 0.41. The longest stretch of contiguous aa was 5. This similarity was evaluated as insignificant. Follow up of this situation is advised since Dermatophagoides sp belong to the most frequently occurring inhalation allergens in moderate climate zones such as in important parts of the US and Europe.

Testing with specific IgE or animal studies were not done (not relevant at this moment).

The author also searched medical databases in order to find reports on allergenicity of the proteins Cry3Bb1 and CP4 EPSPS. No relevant data were found.
In conclusion, it can be stated that at present there is no evidence that the GM maize containing the proteins Cry3Bb1 and CP4 EPSPS will induce allergic reactions. Continuous surveillance is advised. It has also to be taken in consideration that other forms of allergic reactions than IgE mediated are possible (Bernstein et al. 2003).

There are no data indicating that a combination of the above mentioned proteins would increase the allergenic potential.

Comment 2

Assessment of the allergenicity of the introduced traits
The applicant describes Cry1A.105 and Cry2ab2 as non-allergenic on the basis of other documents. However, the fact that Cry1A.105 shows 24.2% identity over 318 aa with actinidin, the major allergen of kiwi (Pastorello et al, 1998), as described in the application for authorization of MON89034 pursuant regulation EC1829/2003, might be a concern. Of course, this identity does not exceed the threshold of 35% over 80 aa, as recommended in the FAO/WHO guidelines, but represents a sufficient number of aminoacids to form common conformational epitopes with actinidin when folded in the 3-D structure, which is not taken into account with single alignment searches. Kiwi allergy is not uncommon in Europe. It might be relevant to perform skin tests with purified Cry1A.105 on kiwi-sensitized patients (the right kiwi-sensitized population must be chosen (Lucas et al, 2007)).
Likewise, it should be mentioned that cross-reactivity of Cry2Ab2 with Cop c 1 (Brander et al, 1999) has also been noticed in the abovementioned application. Basidiomycetes-sensitized patients, however, represent a very small population. To the current knowledge, such cross-reactivity does not appear as an issue.

CP4EPSPS and Cry3Bb1 have been considered as safe by EFSA scientific panel. To the knowledge of the reviewer, there is no new data that could contest this decision.

Assessment of the allergenicity of the whole GM plant or crop
The applicant states: "Therefore a possible over expression of any endogenous protein, which is not known to be allergenic, would be unlikely to alter the overall allergenicity of the whole plant or the allergy risk for consumers". The reviewer feels that it is more of a concern if over-expression of endogenous proteins known to be allergenic occurs, due to the introduction of the four new traits described in the application. Some maize allergens have been described in the literature (Pastorello et al. 2003; Pasini et al. 2002, Weichel et al. 2006), although maize is not considered as a major allergen

source. Therefore, it might be relevant to analyze whether the expression levels of known maize allergens is increased in genetically modified MON 89034 × MON 88017 maize grains. Patient IgE binding to maize grain extract or titration of known major allergens of maize can be carried out.

Summary of above comments as submitted on the EFSAnet

Assessment of the allergenicity of the introduced traits

The applicant describes Cry1A.105 and Cry2ab2 (MON 89034) as non-allergenic on the basis of other documents. However, the fact that Cry1A.105 shows 24.2% identity over 318 aa with actinidin, the major allergen of kiwi (Pastorello et al, 1998), as described in the application for authorization of MON89034 pursuant regulation EC1829/2003, might be a concern. Of course, this identity does not exceed the threshold of 35% over 80 aa, as recommended in the FAO/WHO guidelines, but represents a sufficient number of aminoacids to form common conformational epitopes with actinidin when folded in the 3-D structure, which is not taken into account with single alignment searches. Kiwi allergy is not uncommon in Europe. It might be relevant to perform skin tests with purified Cry1A.105 on kiwi-sensitized patients (the right kiwi-sensitized population must be chosen (Lucas et al, 2007)).

In addition, the following remarks are sound additions to the rationale used by the applicant; they justify the necessity a of post marketing surveillance

- The rapid digestibility of a protein does not warrant non-allergenicity; some labile proteins are allergenic (eg. Mal d 1 form apple)
- The quantity of the protein in food is not absolutely related to allergenicity: allergic reactions can be induced by minute amounts of allergen.

CP4EPSPS and Cry3Bb1 (MON 88017) have been considered as safe by EFSA scientific panel. To the knowledge of the reviewer, there is no new data that could contest this decision. However a follow up is advised since:

- protein CP4 EPSPS had an alignment of 30.5 % identity with Dermatophagoides farinae Der f 2over 82 aa with a high E score of 0.41. The longest stretch of contiguous aa was 5. This similarity was evaluated as insignificant. Follow up of this situation is advised since Dermatophagoides sp belong to the most frequently occurring inhalation allergens in moderate climate zones such as in important parts of the US and Europe.

- protein Cry3Bb1 showed some similarity with the Anisakis simplex tropomyosin Ani s3. The overlap of 120 aa contained four gaps and showed 27.5 % identity with an E score of 1.1. The longest stretch of continuous aa was 3; this was considered as non significant. Tropomyosin is to be considered as pan-allergen in a high number of living animal, with possible cross reactivity (Ebo and Stevens 2001).

Assessment of the allergenicity of the whole GM plant or crop

The applicant states: "Therefore a possible over expression of any endogenous protein, which is not known to be allergenic, would be unlikely to alter the overall allergenicity of the whole plant or the allergy risk for consumers". The reviewer feels that it is more of a concern if over-expression of endogenous proteins known to be allergenic occurs, due to the introduction of the four new traits described in the application. Some maize allergens have been described in the literature (Pastorello et al. 2003; Pasini et al. 2002, Weichel et al. 2006), although maize is not considered as a major allergen

source. Therefore, it might be relevant to analyze whether the expression levels of known maize allergens is increased in genetically modified MON 89034 × MON 88017 maize grains. Patient IgE binding to maize grain extract or titration of known major allergens of maize can be carried out.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1

No further comment taking into account the conclusions with respect to comparative compositional analysis.

D.7.11 Post-market monitoring of GM food/feed

Comments/Questions of the expert(s)

D.8. MECHANISM OF INTERACTION BETWEEN THE GM PLANT AND TARGET ORGANISMS (IF APPLICABLE)

Comments/Questions of the expert(s)

Comment 1

Cultivation is out of the scope of this application. In case of accidental release in the environment, no environmental risk may be expected due to interaction between the GM maize and the target insects (if present).

D.9. POTENTIAL CHANGES IN THE INTERACTIONS BETWEEN THE GM PLANT WITH THE BIOTIC ENVIRONMENT RESULTING FROM THE GENETIC MODIFICATION

D.9.1. Persistence and invasiveness

Comments/Questions of the expert(s)

Comment 1

The GM hybrid maize may be regarded as equivalent to conventional maize varieties, excepted for the Lepidopteran and Coleopteran resistance traits introduced. These traits are not considered as potentially modifying the persistence and invasiveness of maize plants, which are known to be very low in the EU conditions and due to inherent characteristics unrelated to insect susceptibility.

Comment 2

Provided information: sufficient.

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

Comment 1

Same comment as under D.9.1

Comment 2

Provided information: sufficient.

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

Comment 1

Same comment as under D.6

Comment 2

Provided information: sufficient.

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

Comment 1

Cultivation is out of the scope of this application. In case of accidental release in the environment, no adverse effect is expected from the introduced traits, separately or in combination.

Comment 2

Not applicable

D.9.5 Interactions of the GM plant with non-target organism

Comments/Questions of the expert(s)

Comment 1

Cultivation is out of the scope of this application. In case of accidental release in the environment, no adverse effect is expected from the introduced traits, separately or in combination.

Comment 2

Provided information: sufficient.

D.9.6 Effects on human health

Comments/Questions of the expert(s)

Comment 1

Cultivation is out of the scope of this application. In case of accidental release in the environment, no adverse effect is expected from the combination of the traits in comparison with those that would be associated with the single traits. In conclusion, it should be referred to the ERA of the single events (allergenicity, toxicity).

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1

Cultivation is out of the scope of this application. In case of accidental release in the environment, no adverse effect is expected from the combination of the traits in comparison with those that would be associated with the single traits. In conclusion, it should be referred to the ERA of the single events (allergenicity, toxicity).

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

Comment 1

Cultivation is out of the scope of this application. In case of accidental release in the environment, no adverse effect is expected from the combined traits.

Comment 2

Provided information: sufficient.

D.9.9 Impacts of the specific cultivation, management and harvesting techniques

Comments/Questions of the expert(s)

Comment 1

Cultivation is out of the scope of this application. In case of accidental release in the environment, no impact on crop management is to be expected.

Comment 2

Not applicable

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

Comment 1

Cultivation is out of the scope of this application. In case of accidental release in the environment, no adverse effect is expected from the combined traits.

Comment 2

Provided information: sufficient.

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

Comment 1

Cultivation is out of the scope of this application. No environmental risk has been identified, whether associated with the individual traits – to be confirmed by the ERA of the single events – or with the combination of the traits, hence no case-specific monitoring has to be envisaged. As regards general surveillance, the proposed measures comply with the EFSA guidelines and are in line with approved general surveillance plans approved for previous dossiers of the same notifyer, in particular the NK603 glyphosate-tolerant maize evaluated under directive 2001/18 part C.

Comment 2

We support the recommendation of ACRE (2006) that provision of detailed arrangements for general surveillance post-market monitoring plans for the import and processing of grain from GM maize

should be made a condition of any consent. These should include which and when information should be provided to EFSA and how the applicant can ensure this to happen.

Although resistance to insect attack is not the only factor preventing maize to grow outside the agricultural environment, the (indeed low) possibility of the establishment of maize protected against insect larvae in the wild in Europe should be a point of particular interest in a more detailed general surveillance plan.

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

Comment 1

See general remark under D.11.1

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

Comment 1

See general remark under D.11.1

D.11.4 General surveillance of the impact of the GM plant

Comments/Questions of the expert(s)

Comment 1

See general remark under D.11.1

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

Comment 1

See general remark under D.11.1

ADDITIONAL COMMENTS

Comment 1

General Comments/Questions related to **Part II – Summary (SNIF)**

The summary should be as clear as possible and stand on its own. Therefore any lack of clarity should be avoided, especially because this document is also publicly available on the EFSA website. It is the

entire responsibility of the applicant to provide the data/report in such away that no interpretation other than the correct one is possible. Therefore additional info making this part of the dossier more clear should be included.

Here are some suggestions EFSA might transfer to future applicants in order to improve the value of their documents:

- **Section C., pg 10 of the summary**

"*The plasmid vector PV-ZMIR245, used for the transformation of maize cells to produce MON 89034, contains two T-DNAs. T-DNA I includes the cry1A.105 and the cry2Ab2 expression cassettes, while T-DNA II includes the nptII expression cassette... ...The T-DNA I region containing the cry1A.105 and cry2Ab2 gene expression cassettes is the portion of plasmid PV-ZMIR245 maintained in MON 89034.*"

If T-DNA II is segregated from the final selected lines, why is this not mentioned in the text? Based on the actual text for a non-expert it might seem that this T-DNA just has vanished, without any explanation.

- **Section D.2.d), Figure 2., pg 15 of the summary.**

This schematic representation only show a portion of border sequence at the left end side of the T-DNA. Wouldn't it be valuable to explain why a border sequence at the right end side is missing?

- **Section D.3.b), pg 16 of the summary**

Why is the name of the line (MON 88017) always coupled to the Cry3Bb1 protein when referring to the expression level of this protein? Can't the MON 88017 be omitted in front of Cry3Bb1 (throughout the document)? If not, why not? This is especially confusing when referring to the expression level in the hybrid: i.e. the mean MON 88017 Cry3Bb1 protein levels in MON89034 x MON88017.

Can the abbreviations used (OSL, OSR, OSWP), be explained?

Wouldn't it be better to use the word 'location' instead of 'site' (might be confused with integration site – though irrelevant in this context)

- **Section D., pgs 15, 16 & 18 of the summary**

Can ILSI be written in full, at least once, in the text?

Is it correct that the hybrid has been grown in 2005 to verify expression levels and in 2004 to generate material for toxicity and allergenicity tests?

- **Section E, 2. a) and e)**

If the hybrid is grown since 2004 in the USA (as stated in a) of this section and on pgs. 15 and 16), why is then mentioned under e) that the duration of the release is only 12 months in USA/Argentina? This seems inconsistent and seems to be at least 24 months in the USA (12 months of 2005 for expression level material and 12 months for toxicity/allergenicity material in 2004).

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