Bioveiligheidsraad
Conseil de Biosécurité

17-12-2010

Secretariaat
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O./ref.: WIV-ISP/41/BAC/2010_1159[1]

Title: Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/CZ/2008/62 from Dow AgroSciences and Monsanto under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/CZ/2008/62 was submitted by Dow AgroSciences and Monsanto on 28 October 2008 for the marketing (import and processing) of the insect resistant and glyphosate/glufosinate-tolerant genetically modified MON89034 x 1507 x MON88017 x 59122 maize for food and feed uses under Regulation (EC) No. 1829/2003[2].

The application was officially acknowledged by EFSA on 3 March 2009. 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 (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 Biosafety Advisory Council and the Division of Biosafety and Biotechnology (SBB). Ten 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 2 June 2009.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 8 September 2010 (The EFSA Journal, 2010, 8 (9):1781)[3], and published together with the responses from the EFSA GMO Panel to comments submitted by the experts during the three-month consultation period.

On 29 September 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. In addition, the complementary information sent by the company to EFSA after 4 June 2009 was provided to the coordinator and to the sole experts who evaluated the toxicological aspects of this GM maize. The comments formulated by the experts together with the opinion of EFSA including the answers

[1] Revised version completed with minority declaration
[2] 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)
[3] See: http://www.efsa.europa.eu/en/scdocs/scdoc/1781.htm

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of the EFSA GMO Panel form the basis of the advice of the Biosafety Advisory Council given below.
In addition, the scientific evaluations of the single events, namely maize line MON89034 (EFSA/GMO/NL/2007/37), maize line 1507 (EFSA/GMO/NL/2004/02 and EFSA/GMO/RX-1507), maize line MON88017 (EFSA/GMO/CZ/2005/27) and maize line 59122 (EFSA/GMO/NL/2005/12), are taken into account in this advice. The Biosafety Council formulated a positive advice for each single event but line 1507. For this latter, due to the lack of quality of animal trials for toxicity testing and testing of the nutritional value provided by the applicant the Biosafety Advisory Council did not to draw conclusions about the feed safety of this GM maize.[4].

Scientific evaluation

1. Environmental risk assessment

According to the Biosafety Advisory Council no major risks were identified concerning the environment[5].

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
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.

Following the comments submitted by the Belgian experts, the Biosafety Advisory Council considers that even if the compositional analysis of the GM food/feed was performed according to the OECD consensus document[6], it lacks the analysis on dietary fibre. The Biosafety Advisory Council recommends the analysis on dietary fibre since this concept is widely accepted in human food studies and recommends the adaptation of the OECD consensus documents accordingly.

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. No allergenicity assessment was performed on the whole GM maize. With regard to allergenicity, the Biosafety Advisory Council is of the opinion that the information provided is sufficient and does not raise safety concerns.

[4] Advice of BAC on maize line MON89034: BAC_2009_0880; Advice of BAC on maize line 1507: BAC_2009_01368; Advice of BAC on maize line MON88017: BAC_2009_01045; Advice of BAC on maize line 59122: BAC_2007_SC_536;
[5] 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.
[6] OECD, 2002. Consensus Document on Compositional Considerations for New Varieties of Maize (*Zea mays*): Key Food and Feed Nutrients, Anti-Nutrients and Secondary Plant Metabolites. ENV/JM/MONO(2002)25. http://www.olis.oecd.org/olis/2002doc.nsf/LinkTo/env-jm-mono(2002)5

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

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.

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

"maize MON89034 x 1507 x MON88017 x 59122 is unlikely to have adverse effects on human and animal health and the environment, in the context of its intended uses".

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

The Biosafety Advisory Council also recommends EFSA to take into account our remarks addressed in document WIV-ISP/41/BAC/2010_0902 of 21 September 2010 (see Annex III).

p.o. M. Ph. HERMAN

Prof. D. Reheul

President of the Belgian Biosafety Advisory Council

Annex I : Minority declaration

Annex I: Full comments of experts in charge of evaluating application EFSA/GMO/CZ/2008/62 *(ref. BAC_2009_949)*

Annex II: Comments submitted on the EFSAnet for application EFSA/GMO/CZ/2008/62 (ref. BAC_2009_950)

Annex III: List of issues on risk assessment of GMOs, sharing best practice, etc. that the Biosafety Advisory Council and/or the SBB would like to propose for discussion at the first and subsequent meetings of the EFSA Scientific Network (ref. BAC/2010_0902)

Annex I – Minority declaration of L. Flandroy (22/12/2010)

Following the data available on this file, I would rather have formulated (as a member of the Belgian Biosafety Consultative Council - BAC) the following conclusion of the BAC on this file:

" ….. Maize MON89034 x 1507 x 59122 is unlikely to have **short term** adverse effects on human and animal health in the context of its intended uses. …… N.B.: special caution should be taken for individuals having susceptibility to maize allergy ……."

Indeed, no sub-chronic (90 days feeding tests) or long-term (on reproductive functions, for ex.) nutritional/toxicological assessment was performed, in contradiction with the EU legislation.

A 42-day nutritional/toxicity study of the whole plant was performed (only on chickens, measuring only a very limited number of physiological parameters, and leaving some uncertainty regarding safety: higher mortality and heterogeneity of results in GM fed group, maybe linked to a badly designed experiment – not enough animals, statistical power of experiments too low – ; reflected in the recommendation of the BAC in the Conclusions of its advice).

In any case, I do not agree that a short-term nutritional/toxicological study is enough to be able to conclude in long-term safety, neither that compositional analysis' comparison between the GMO and the control(s), especially as performed presently, is a valuable analysis to draw conclusions on potential long-term effects of GMOs (see also minority advice on file EFSA/GMO/NL/2009/65 for other details): the present analytical composition study requirements do not include, e.g., dosage of potential carcinogenic compounds present in maize (hydroxamic acids and derivative compounds, some of them having toxicological/mutagenic potential), neither an up-to –date analytical method for carbohydrates (allowing to define the composition of carbohydrates now that "*more and more attention is given to the type of carbohydrates present in human food"*) and fibers (allowing to distinguish between soluble and insoluble fibers, having different digestibility and physiological effects) (cf. document of Compilation of comments of BE experts on this file p. 8-9; cf recommendation of the BAC in the Conclusion of its advice; cf. written messages addressed by the BAC to EFSA relatively to these questions in June 2007, June 2008, September 2010 – this time also for hydoxamic acids and derivatives- , without real taking into account by EFSA till now of the preoccupations of the BAC).
EFSA' s recently reviewed guidelines on this matter confirm my view: it states that if there are remaining uncertainties relative to risks, a.o. based on compositional analysis (which is the case in absence of adequate compositional analysis), a 90-day study in rodents should be performed.

(The uncertainties in this file on health risk assessment cannot be enlightened by a toxicity study of the isolated combined proteins coded by the transgenes of the stacked event, that was not performed on the basis of the improbability of interaction between them, as suggested by their mode and site of action. In any case, it is recognized by EFSA that unintended and unexpected changes can occur in the GM plants beside those directly induced by the intended changes).

Concerning **allergenicity**: whereas maize is not officially considered as an allergenic plant by concerned OECD documents dated from several years, allergenic proteins in maize have now repeatedly been described in the scientific literature, even able to provoke anaphylactic reactions by ingestion (susceptible to kill), as mentioned by 1/3 Belgian experts assisting the BAC in this file (cf. p. 20 of Compilation of comments of experts, with reference to scientific articles) . Whereas those reactions are rare and mostly provoked by maize pollen (normally out of scope of the present file for food/feed import and no cultivation), the unintended changes induced by the transgenic modification and the stacking of transgenes, one of them coding for a protein having ~ 25 % sequence similarity with a known allergen (Cry1A.105 of MON89034: similarity with Kiwi allergen) could enhance the allergenic potential of maize (N.B. : the potential allergenicity of non of the single genes has been tested). As repeatedly suggested by a BE expert, easy tests like dosage of known maize allergenic proteins and measurement of reaction of the serum (more precisely, IgE binding tests) of patients allergic to maize or to those allergens having homology with the "tranproteins", or cutaneous sensitization of patients allergic to those known allergens with GM maize grain extracts, could be performed to give useful indications on the potential enhanced allergenic character of those stacked genes varieties, even if not being ideal conclusive tests, which are note easy to draw due to the rather individual and rare aspect of immunological reactions. The public should be informed of this potential allergenic problem, if no valuable risk assessment is made.

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May 2009

Compilation of comments of experts in charge of evaluating the application EFSA/ EFSA/GMO/CZ/2008/62

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 17 March 2009
Coordinator: Prof. dr. ir. Dirk Reheul
Experts: Pascal Cadot (Consultant), Armand Christophe (UGent), Leo Fiems (ILVO), Rony Geers (KUL), André Huyghebaert (UGent), Peter Smet (Consultant), Wim Stevens (UA), Jan Van Doorsselaere (KH Zuid-West Vlaanderen), Hadewijch Vanhooren (KUL), Michel Van Koninckxloo (HEPHO)
Domains of expertise of experts involved: Genetics, molecular characterisation, human nutrition, animal nutrition, analysis food/feed, traceability of alimentary chain, substantial equivalence, toxicology in vitro, in vivo, general biochemistry, immunology, alimentary allergology, agronomy, agro-ecology, ecotoxicology, herbicide tolerance
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

INTRODUCTION

Dossier **EFSA/GMO/CZ/2008/62** concerns an application of the company **Monsanto & Dow AgroSciences** for the marketing of the genetically modified **maize MON89034 x 1507 x MON88017 x 59122** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 3 March 2009.
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).
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.

List of comments received from the experts

GENERAL COMMENTS

Comments/Questions of the expert(s)

Comment 1

Serious problems are not really expected by the application of MON89034 x 1507 x MON88017 x 59122 maize because:

- The lines that constitute this new hybrid has already received a safety evaluation
- Maize has a safe history of use for human food and animal feed
- The concentrations of the newly expressed proteins are low

However, the safety aspects of the different newly inserted proteins in MON89034 x 1507 x MON88017 x 59122 maize is assumed based on the safety aspects of the individual new proteins. The safety aspects of the multiple challenge, due to the combination of the newly inserted proteins, are rather weakly demonstrated.

Comment 2

MON 89034 × 1507 × MON 88017 × 59122 was obtained by conventional breeding of four single event products: MON 89034, 15072, MON 88017 and 591223.

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1

The information given is adequate. It is important to note that the safety of MON 89034 and MON 88017 is still under scientific review by the EFSA GMO panel (Technical dossier I, page 14).

Comment 2

No comments

Comment 3

The information provided in the application is sufficient.

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

Comments/Questions of the expert(s)

Comment 1

The information given is adequate.

Comment 2

No comments

Comment 3

The information provided in the application is sufficient.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Comment 1

P31; could the authors state why this construct also contains –what it seems- partial cry1F and pat fragments?
What is the size is of these fragments and why is hybridisation not seen in Southern blots?

Comment 2

The information provided in the application is sufficient.

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

No comments

Comment 2

The information provided in the application is sufficient.

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

Comments/Questions of the expert(s)

Comment 1

P36: is it possible to add data on the chromosome location of the four inserts (and segregation analysis in relation with the breeding scheme in fig 13 p51)?

P39: what can be said about the band of 4.2 Kb? Is this due to partial restriction of genomic DNA?

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1

Question : Is there no contradiction between the statement "… the levels of Cry1A.105….are comparable to the protein levels in the positive controls…" (Technical dossier, part I, page 45) and the data provided in Table 6 for this protein (4.3 vs 2.8 in the control)? (almost no overlap in range; means are about 3 SD different).

Comment 2

P45 I am not sure whether statistically there is a difference in Pat levels between 59122 and (89034 x 1507 x 88017 x 59122) as stated and therefore to my opinion the sentence: "This is likely due to the presence of multiple copies of the pat gene…" should be deleted.

Comment 3

Comment : In part 1 of the technical dossier we can read p.45 "For the PAT protein, expression was higher in the combined trait product as compared to 1507 and 59122" but the Table 12 (PAT) shows that the values for PAT protein levels in grain collected from MON 89034 × 1507 × MON 88017 × 59122 (0.050 µg/g dw) are similar to those of 59122 (0.049).

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

No comments

Comment 2

The information provided in the application is sufficient. (The scope of the application does not include authorization for the cultivation of 98140 maize seed products in the EU).

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

Comments/Questions of the expert(s)

Comment 1

Although the new hybrid maize seed will not be marketed for breeding (Technical Dossier I, page 62), can this practise be excluded?

Comment 2

No comments

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

No comments

Comment 2

The information provided in the application is sufficient.

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

Questions

1) Table 21, Technical dossier, part I, page 73. Is there no mistake in the reported mean value for linoleic acid in the control grain? This value seems unlikely to me (for a non high-oleic acid corn oil).

2) The presence of trypsin and chymotrypsin inhibitors have been described in corn (Shulima et al., 1985). No data are reported on the level of these antinutrients.

Comments
The slightly reduced level of Vitamin B1 and the slightly increased level of stearic acid in the new hybrid oil compared to control are of no nutritional importance.
Minor comments
1) Palmitoleic acid is not mentioned in table 20 of the Technical Dossier, part I, page 69. Yet it is known to be a minor component in corn oil and was found in some positive controls. The claim N/A (not available) is unlikely as the fatty acid composition was determined by gas chromatography. This omission is of no nutritional importance however.
2) It is not clear what is meant with “maize tissues” in the second paragraph on page 65 of Technical Dossier, part I. (…maize tissues that are consumed??) Printing error?

Comment 2

Results of the maize sample analyses showed that the levels of key nutrients, anti-nutrients, and secondary metabolites quantified in forage and grain of MON 89034 × 1507 × MON 88017 × 59122 and XE6100 are representative of those in conventional maize. The few observed differences between test and control were not regarded as biologically relevant because all values fell within the range of values for the reference substances analyzed in this study and within the range of values found in the literature and/or in the ILSI database.

Comment 3

The approach is quite in line with previous dossiers.
The OECD document was followed for the comparative analysis of the maize MON89034 x 1507 x MON88017 x 58122 and a conventional maize with similar genetic background. As a reference fourteen conventional maize hybrids were included in the study.
With respect to nutrients 69 are included for grain and 9 for forage.
Results were statistically analyzed to detect any significant difference.

In most cases no difference was observed. The ILSI databank for comparison is used in case any significant difference is found. Comparisons always include a combined site analysis and an individual site analysis.

No differences were found for proximates and fibre with the exception of the moisture content in two sites and the protein content in one site.
I agree with the comment of the applicant that these differences are not biologically relevant.

In the area of amino acids, fatty acids, anti-nutrients and secondary plant metabolites in grain no differences were detected in the combined site analysis with one exception, stearic acid. The values obtained were however in the range of values for this constituent in the ILSI database and considered as not relevant.
In the individual site analysis differences in fatty acid composition were observed at one site. Values are within the range of literature data.

No differences were detected for anti-nutrients and secondary plant metabolites. The applicant makes some comments on the importance of phytic acid, ferulic and p-coumaric acid.
I agree with these comments. In my opinion this is a good example of a comparative assessment beyond traditional nutrients with attention for constituents of growing nutritional importance.

In the field of minerals and vitamins no differences were found in the combined site analysis with the exception of vitamin B1. The values obtained are however within the range of literature data.
Additional differences were found in the individual site analysis for copper, magnesium, manganese and zinc in grain, for phosphorus in forage and for vitamin B2, vitamin E and folic acid in grain. Values are however in the range of literature data.

The applicant concludes that with respects to key nutrients, anti-nutrients and secondary plant metabolites, grain and forage of the maize MON89034 x 1507 x MON88017 x 58122 are compositionally equivalent to conventional maize.

Taking into account the in-depth analysis of the composition, the selection of the samples and the statistical analysis I agree with this conclusion.

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

The production of material for comparative assessment is according to the guidelines.

Comment 2

The maize MON89034 x 1507 x MON88017 x 58122, the reference and commercially hybrids were grown at five locations in the US.
No further questions

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

The selection of the compounds for analysis is according to the OECD consensus document 6 (2002). However, considering that DIMBOA and its glycoside may total 1% of dry weight in conventional corn plants (Klun et al.,1969) and that mutagenic effects in human cell lines have recently been demonstrated (Buchmann et al., 2007), it seems of value to determine these components in corn containing stacked events. Hormonal effects of its degradation product MBOA have been described in rodents (in OECD report 6, page 28).

It is claimed that 2-furaldehyde was determined (Technical Dossier I, page 82). Yet, I have found no data on levels of this component. Did I miss them or were they not reported? Although furfural has GRAS status, it has been suggested that an increase of the furfural level in food stuff should be avoided and that furfural is considered as a dietary risk factor for cancer (Feron et al.,1991).

Comment 2

The information provided in the application is sufficient.

Comment 3

Grain and forage are analyzed for nutrients, according to the OECD documents. In addition other constituents with growing importance are included in the study.
On the other hand a rather traditional approach was chosen for the proximate nutrients. No information is available on the composition of the carbohydrate fraction as it is calculated “by difference”. This is regrettable from a nutritional point of view as more and more attention is given to the type of carbohydrates present in human food.
The same remark further applies for the fibre fraction, as mentioned several times before in previous dossiers.
The OECD document needs to be adapted to current knowledge in human nutrition (see also EU definition of fibre).

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient. (The scope of the application does not include authorization for the cultivation of 98140 maize seed products in the EU).

D.7.5 Product specification

Comments/Questions of the expert(s)

Comment 1

No questions.

Comment 2

The information provided in the application is sufficient.

Comment 3

No comments

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1

No questions.

Comment 2

The information provided in the application is sufficient.

Comment 3

Due to the compositional equivalence of the maize MON89034 x 1507 x MON88017 x 58122 to commercial available maize, characteristics of processing and processed product will not be affected. The applications of maize in animal feed and the processing into food and industrial products like ethanol, by dry milling, wet milling and fermentation are reviewed.
The applicant states that the product obtained by processing are expected to be equivalent to the products obtained from traditional maize.
I agree with this conclusion.

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

No questions.

Comment 2

The information provided in the application is sufficient.

Comment 3

No comments

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

In the case of stacked events, it is advisable to perform an animal feeding study with relevant foods/feeds derived from the stacked GM plant. This was done in broiler chickens and no adverse effects were observed.

Comment 2

MON 89034 x 1507 x MON 88017 x 59122 maize has been obtained from traditional breeding methods between four genetically modified maize. No new genetic modifications have been introduced in MON 89034 x 1507 x MON 88017 x 59122 maize. The parental lines contain the genes Cry1A.105 and Cry2Ab2 (MON 89034), Cry1F (1507), Cry3Bb1 (MON 88017), Cry34Ab1 and Cry35Ab1 (59122), CP4 EPSPS (MON 88017), and PAT (1507 / 59122) conferring resistance to certain lepidopteran and coleopteran insects and tolerance to herbicides containing the active ingredient glufosinate-ammonium or glyphosate. All the introduced traits from the parental lines are inherited in MON 89034 x 1507 x MON 88017 x 59122 maize. This results in the combined expression of the cry, pat, and cp4 epsps proteins in the same plant. The lines 1507, 59122, and MON 89034 are already authorized (line 1507: 2006/197/EC; line 59122: 2007/702/EC; line MON 89034: 2008/909/EC).

Comment 3

P.63 of the Technical Dossier: Fourteen conventional maize hybrids were included as reference for the toxicological and allergenic effects: is it possible to mention these hybrids?

A detailed description of the safety aspects of the Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS proteins is given in the corresponding toxicology section of the respective applications for authorisation of MON 89034, 1507, MON 88017 and 59122. In case of MON 89034 × 1507 × MON 88017 × 59122 there may be a multiple challenge, which can be more harmful than any individual newly inserted proteins. It is highly desirable to refer to studies that have demonstrated that the combination of all these newly inserted proteins is not detrimental.
According to the Ouellet et al. (2003) the TMR contained 0.446 maize silage (containing approximately 70 g protein/kg dry matter; DM) and 0.188 cracked maize (containing approximately 100 g protein/kg DM), resulting in 40 g maize protein/kg dietary DM. However, DDGS (containing approximately 300 g protein/kg DM), the by-product from the bioethanol production is sometimes incorporated in diets for dairy cows at 0.3 - 0.4 (Hippen et al, 2003; Kalscheur et al., 2004; Janicek et al., 2008), and distillers grains were be used at 50% in diets for finishing beef cattle (Roeber et al., 2005). Diets with 40% DDGS may yield ±120 g maize protein/kg dietary DM, which means that:

- the protein amount coming from maize, via DDGS, is 3 x higher than the protein amount coming from directly from MON 89034 × 1507 × MON 88017 × 59122 grain and/or silage
- this protein also contain about 3 x more newly inserted protein

What is the effect of such a diet on the safety for the animals?

However, the animal feeding study, reported by Davis (2008) and conducted with relatively high incorporation levels in the diets of broilers, did not show health problems which can be attributed to this MON 89034 × 1507 × MON 88017 × 59122 maize, which is an indication of a safe use.
Nevertheless, some caution is desirable. A study of Séralini et al. (2007) revealed signs of hepatorenal toxicity in rats due to the genetically modified Maize MON 863, which contains a variant of the Bacillus thuringiensis Cry3Bb1 gene. MON 89034 × 1507 × MON 88017 × 59122 produces Cry3Bb1 insecticidal protein and may therefore be similar to MON 863. Consequently, MON 89034 × 1507 × MON 88017 × 59122 may be suspicious, on the one hand because of its similarity with MON 863, and on the other hand because of the combined challenge of newly inserted proteins.

Comment 4

Protein levels measured in MON 89034 × 1507 × MON 88017 × 59122 maize grain

Protein	**ng/mg Tissue Dry Weight**		**Standard deviation**
	Mean	**Range**	
Cry1A.105	4.3	3.4 – 4.9	0.44
Cry2Ab2	5.7	4.1 – 7.5	0.94
Cry1F	3.34	2.12 – 7.43	1.23
Cry3Bb1	18	10 - 26	4.6
Cry34Ab1	62.5	47.8 – 94.0	11.4
Cry35Ab1	1.69	1.24 – 2.31	0.28
PAT	0.050 (between LOD and LOQ)	ND – 0.10	0.023
CP4 EPSPS	5.2	3.5 – 7.1	1.1

These data show that the levels of Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab2 and CP4 EPSPS proteins in grain of MON 89034 × 1507 × MON 88017 × 59122 are comparable to protein levels in the positive controls substances, MON 89034, 1507, MON 88017 and 59122, as appropriate.

The introduced proteins are expressed in leaf, root, forage, pollen and grain at appropriate times of plant development (Phillips, 2008; Stillwell and Silvanovich, 2007a).

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

No questions.

Comment 2

No new genetic modifications have been introduced in MON 89034 x 1507 x MON 88017 x 59122 maize.
The safety of the proteins Cry1A.105, Cry2Ab2, Cry1F, Cry34/35Ab1, and PAT have already been confirmed in detail in accordance with the applications for authorisation of 1507, 59122, and MON 89034 maize:
1507: Cry1F, PAT
Safety confirmed by acute oral toxicity testing and degradation in simulated digestive fluids.
59122: Cry34Ab1, Cry35Ab1, PAT
Safety confirmed by acute oral toxicity testing, repeated oral toxicity testing (28-d), and degradation in simulated gastric fluids, bioinformatic studies.
MON 89034: Cry1A.105, Cry2Ab2
Safety confirmed by acute oral toxicity testing, degradation in simulated digestive fluids, and bioinformatic studies.
MON 88017: Cry3Bb1, CP4 EPSPS
Reference to the evaluation of the EFSA dossier EFSA/GMO/CZ/2005/27:
Similar proteins to the two proteins present in MON 88017 maize have been assessed previously for safety (MON 863, NK603). Additionally, a battery of tests designed to evaluate the Cry3Bb1 variant protein and the native CP4 EPSPS protein present in MON 88017 maize for characteristics associated with food allergens and toxins raised no concern. The mature CP4 EPSPS in MON 88017 is identical to the bacterial enzyme of 455 amino acids and is targeted to the plant chloroplast. The Cry3Bb1 in MON 88017 differs from the native Cry3Bb1 by 6 amino acid changes, and differs from the in MON 863 variant by only 1 amino acid. Both novel proteins are expressed at relatively low levels in MON 88017.
CryBb1
No adverse effects were observed when Cry3Bb1 protein was ingested by mice at a dose of 1930 mg/kg bw. Bioinformatic studies confirmed the absence of any significant amino acid similarity with known toxins and allergens. In vitro digestibility studies demonstrated that the Cry Bb1 variant was rapidly degraded in simulated gastric fluid. Furthermore, the Cry Bb1 variant is not glycosylated in maize. Processing involving heat treatment rendered the CryBb1 variant protein non-functional.
The CryBb1 variant protein used in the studies was obtained in an *E. coli* production system. The equivalency of the MON 88017 maize produced protein to the *E. coli*- produced protein was evaluated by comparing the molecular weight, immunological reactivity, insecticidal activity and glycosylation. Both proteins were found to be equivalent.
CP4 EPSPS
In previous assessments (e.g. NK603), a battery of tests designed to evaluate the CP4 EPSPS protein for characteristics associated with food allergens and toxins raised no concern. The CP4 EPSPS protein shared no sequence homology with known toxins. There is a rapid digestion of the CP4

EPSPS protein in simulated digestive conditions, susceptibility to heating, and lack of acute toxicity for the CP4 EPSPS protein as determined by the mouse acute oral toxicity study.
The CP4 EPSPS protein used in these studies was obtained in an *E. coli* production system. The equivalency of the MON 88017 maize produced protein to the *E. coli*- produced protein was evaluated by comparing the molecular weight, immunological reactivity, glycosylation and functional activity. Both proteins were found to be equivalent.

Potential interactions of the introduced Cry proteins produced by MON 89034 x 1507 x MON 88017 x 59122 by Insect Bioassay (Levine, 2008): no interactions among the *Bt* Cry proteins expressed in MON 89034 x 1507 x MON 88017 x 59122.

Comment 3

Safety assessment of these proteins has been extensively performed in earlier notification dossiers.

The only question I have concerning this item is whether an up-to-date sequence homology search has been performed for each of the proteins?

Interaction between the different proteins?
The modes and sites of biological activity are different for the Cry, PAT and CP4 EPSPS proteins and there is no known or conceivable mechanism of interaction between these proteins which could lead to adverse health effects in animals or humans.

Potential interactions of the introduced Cry proteins in target insect species
The presence of different specific receptors for the various Cry proteins limits the potential for interactions between these proteins (Hofmann et al., 1988b; Hofte et al., 1988; Lambert et al., 1996; OECD, 2007; Pigott and Ellar, 2007; Rose and Dively, 2007; Van Rie et al., 1989; Van Rie et al., 1990). This may explain the fact that there have been only a few reported examples of interactive effects between Cry proteins that have either decreased (antagonism) or increased (synergism) the combined insecticidal activity towards target pests (Schnepf et al., 1998; Tabashnik, 1992).

To assess the potential for interaction between the combination of the Cry1A.105, Cry2Ab2 and Cry1F proteins, European corn borer (ECB) larvae were fed lyophilized leaf tissue at different concentrations of MON 89034 (contains Cry1A.105, Cry2Ab2); 1507 (contains Cry1F) or MON 89034 × 1507 × NK603 (contains the same aforementioned Cry proteins plus CP4 EPSPS) along with the appropriate control tissues in 7-day diet-incorporation bioassays (Levine et al., 2008). Bioassay results indicated no interaction among the three proteins.

To assess the potential for interaction between the combination of the Cry3Bb1 and Cry34/35Ab1 proteins, southern corn rootworm (SCRW) larvae was exposed to different concentration of purified Cry3Bb1 protein, a mixture of purified Cry34Ab1 and Cry35Ab1 proteins and a combination of all three purified proteins in 6-day diet-overlay bioassays (MacRae, 2008). LC50 and GI50 values obtained with this bioassay using the combination of the Cry3Bb1 protein and the Cry34/35Ab1 binary proteins did not show evidence of a significant interaction. Therefore, the results for mortality and growth inhibition are consistent with additivity.

To assess whether combined Cry1A.105, Cry2Ab2 and Cry1F activity is altered by the presence of the Cry3Bb1 and Cry34/35Ab1 proteins, the biological activity of MON 89034 × 1507 × MON 88017 ×

59122 and MON 89034 × 1507 × NK603 were compared (Levine et al., 2008). To make this comparison, ECB larvae were exposed to a series of lyophilized leaf tissue at different concentrations of the two combined trait products along with the appropriate control tissues in 7-day diet-incorporation bioassays. No difference in the biological activity was observed, again indicating no interaction between the insecticidally active proteins.

Comment 3

The information provided in the application is sufficient.

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Comment 1

No questions.

Comment 2

No constituents other than the Cry1A.105, Cry2Ab2, Cry1F, Cry34/35Ab1, Cry3Bb1, PAT, and CP4 EPSPS proteins are novel.

Comment 3

The information provided in the application is sufficient.

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1

No questions.

Comment 2

No particular natural constituents of maize are considered to be of significant concern to require additional information or further risk assessment.
Compositional analysis of forage and grain (Lundry et al., 2007): The few observed differences between test and control were not regarded as biologically relevant because all values fell within the range of values for the reference substances analysed in this study, and within the range of values for commercial corn found in the ILSE database and/or the literature.

Comment 3

The information provided in the application is sufficient.

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

No indications for unanticipated pleiotropic effects linked to the genetic modification were identified in then broiler chicken feeding experiment. Neither was there an indication of adverse effects of feeding the introduced proteins together.

Comment 2

An animal feeding study was conducted using whole-grain MON 89034 x 1507 x MON 88017 x 59122 fed to broiler chickens (Davis, 2008). The results of the well-performed broiler feeding study showed that there were no biologically significant differences on the parameters tested (performance, carcass measurements) between broilers fed MON 89034 x 1507 x MON 88017 x 59122 or the broilers fed conventional control or reference corn.
No additional 90-d rat feeding study using whole-grain MON 89034 x 1507 x MON 88017 x 59122 is requested. Rat feeding studies were already conducted with the individual maize lines (see earlier applications) confirming the absence of any toxic effects associated to the introduced proteins and the absence of any unanticipated or pleiotropic effects linked to the genetic modifications. The well-performed field experiments with agronomic data, the compositional study, and the poultry broilers feeding study provide sufficient confirmation of the safety of MON 89034 x 1507 x MON 88017 x 59122.

Comment 3

a) 49-day feeding study in broiler chickens (Davis, 2008).
From Day 7 - 42 bird mortality averaged 1.9% and ranged from 0 to 4 % across all treatment groups. Mortality from Day 7 - 42 was 1% for birds receiving diets containing MON 89034 × TC1507 × MON 88017 × DAS-59122-7. Apparent causes of death identified at necropsy for most birds that died after Day 7, sudden death syndrome and bacterial infection, occur commonly in chickens.

Performance of broilers fed diets containing the MON 89034 × TC1507 × MON 88017 × DAS-59122-7 corn was not different (P ≥ 0.05) than that of broilers fed diets formulated with conventional control corn grain of similar genetic background. Performance was also not different (P ≥ 0.05) for birds fed diets containing MON 89034 × TC1507 × MON 88017 × DAS-59122-7 compared to the population of birds fed diets containing control and reference corn. Furthermore, performance parameters measured for birds fed diets containing either MON 89034 × TC1507 × MON 88017 × DAS-59122-7 or the conventional control grain were generally within the range of performance observed for birds fed diets formulated to the same nutrient specifications using grain from six conventional reference corn hybrids. No unexpected effects on broiler performance were observed when broilers were fed diets

formulated with MON 89034 × TC1507 × MON 88017 × DAS-59122-7 compared to diets formulated with control or reference corn.

Carcass yield measurements were not different (P ≥ 0.05) for broilers fed diets containing MON 89034 × TC1507 × MON 88017 × DAS-59122-7 compared to those fed diets containing conventional control or reference corn, with **exception of fat pad weight** expressed as kg/bird or % of live bird weight (P < 0.05) (detected difference in fat pad weight for birds fed diets containing MON 89034 × TC1507 × MON 88017 × DAS-59122-7 and conventional control corn). Fat pad weight for birds fed diets containing MON 89034 × TC1507 × MON 88017 × DAS-59122-7 was not different (P ≥ 0.05) from that of birds fed diets containing any of the six reference corn lots. *(So there was a significant difference between the test material and its control, but not between the test material and the reference materials).*
A diet × sex interaction (P < 0.15) was detected for four carcass variables (breast weight expressed as kg/bird, and breast, wing, and drum weight expressed as % of chilled carcass weight). Within sex analyses for these variables detected no difference (P ≥ 0.05) between MON 89034 × TC1507 × MON 88017 × DAS-59122-7 and control (XE6001) for any of the four variables for either male or female birds. For all variables for which a within sex diet effect was detected (P < 0.05), the mean value for birds fed diets containing MON 89034 × TC1507 × MON 88017 × DAS-59122-7 was within the range of values for birds fed diets containing conventional reference corn lots. Carcass yield was not different (P ≥ 0.05) for birds fed diets containing MON 89034 × TC1507 × MON 88017 × DAS-59122-7 compared to the population of those fed diets containing conventional control and reference corn grain. Average carcass measurements for birds fed diets containing MON 89034 × TC1507 × MON 88017 × DAS-59122-7 and the control grain were generally within the range observed for birds fed diets formulated to the same nutrient specifications using six conventional reference corn hybrids.

Measurement of **fat, moisture and protein content** of skinless breast and thigh meat samples collected during bird processing showed no differences (P ≥ 0.05) among dietary treatments. Meat analysis results were not different (P ≥ 0.05) for birds fed diets containing MON 89034 × TC1507 × MON 88017 × DAS-59122-7 versus those of birds fed diets containing conventional control or reference corn based on individual diet comparisons or comparison to the population of control and reference corn diets.

Conclusion:
Two models were used:

- *In appendix III table 3 the different diet groups were compared two by two. Only for the fat pad weight there is a significant difference between the group fed the GMO and the control. In this case the fat pad weight for birds fed diets containing MON 89034 × TC1507 × MON 88017 × DAS-59122-7 was not different (P ≥ 0.05) from that of birds fed diets containing any of the six reference corn lots.*
- *In appendix III table 4 the GM material is compared to the control and reference diets as a group. No significant differences appeared for any of the parameters.*

There were no biologically relevant differences in broiler performance, carcass yield or meat composition between broilers fed diets containing MON 89034 x TC1507 ***x*** *MON 88017 x DAS-59122-7 and those fed diets containing the conventional control corn.*

f) 90-Day rat feeding study ().
Not performed. No further testing is needed.

Comment 4

The information provided in the application is sufficient.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

Note that the safety of MON 89034 and MON 88017 is still under scientific review by the EFSA GMO panel (Technical dossier I, page 14).

Comment 2

It is expected that the chance that MON 89034 × 1507 × MON 88017 × 59122 maize is allergenic is small, based on the applications of the single events.

The rapid digestibility in simulated digestive fluids is not a guarantee for safety. Bannon et al. (2003) and Herman et al. (2006) concluded that the use of the SGF technique to predict the allergenic status of the proteins remains uncertain and Spök et al (2005) have shown that digestibility studies can not be considered as suitable tools to address the allergenic potential of a protein.

Comment 3

According to the company, "the assessment of the allergenic potential of proteins compares the biochemical characteristics of these proteins to characteristics of known allergens. A protein is not likely to be an allergen if:
1. The protein is from a non-allergenic source;
2. The protein does not share structural similarities to known allergens based on the amino acid sequence;
3. The protein is rapidly digested in simulated gastric fluid;
4. The protein represents only a very small portion of the total protein in the grain.
These four characteristics have been discussed in detail for the Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS proteins produced in MON 89034 × 1507 × MON 88017 × 59122, in the applications for authorisation of MON 89034 (Monsanto Company (2006) - EFSA-GMO-NL-2007-37 – Section D.7.9 p.197), 1507 (Pioneer Hi-Bred International Inc. and Mycogen Seeds c/o Dow AgroSciences LLC (2000) - C/NL/00/10 – Section B.41 p.54, MON 88017 (Monsanto Company (2005) - EFSA-GMO-CZ- 2005-27 – Section 7.9 p.150) and 59122 (Pioneer Hi-Bred International Inc. and Mycogen Seeds c/o Dow AgroSciences LLC (2005) - EFSA-GMO-NL-2005-12 – Section 7.9 p.54) in the E.U.
It is important to consider that Cry1F, MON 863 Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS have already been assessed by EFSA and considered as safe for humans and animals (EFSA, 2003a; EFSA, 2003b; EFSA, 2004a; EFSA, 2004b; EFSA, 2004c; EFSA, 2005; EFSA, 2007)
The Cry1A.105, ry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS proteins have been assessed for their potential allergenicity according to the recommendations of Codex

Alimentarius Commission (Codex Alimentarius Commission, 2003). The proteins are from non allergenic sources, lack structural similarity to known allergens, are rapidly digested in simulated gastric fluid, and constitute a very small portion of the total protein present in the grain of Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS. Taken together these data lead to the conclusion that the Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS proteins are unlikely to have any allergenic potential, and MON 89034 × 1507× MON 88017 × 59122 is as safe as conventional maize regarding the risk for allergenicity."

Nevertheless the above cited rules are not absolute:

- a protein or polypeptide inserted in an other protein can end up with conformational changes of the original protein. Allergens are not only linear epitopes but can be formed by conformational epitopes.
- The rapid digestibility of a protein does not garantee 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

The assessment of allergenicity of the whole GM plant or crop was evaluated as follows:
"Maize is not considered a common allergenic food. Food allergies to maize are of low frequency and mainly occur in populations of specific geographic areas. Rare cases of occupational allergy to maize dust have been reported. There is no reason to expect that the use of MON 89034 × 1507 × MON 88017 × 59122 will significantly increase the intake and exposure to maize. 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."

In conclusion:
The classical evaluation methods have been used and do not demonstrate the GMO to be a product which might be associated with allergy development. However, since the methods used are not completely predictive for allergy development long term follow up is warranted.

Comment 4

Assessment of the allergenicity of the newly expressed proteins.
According to currently available data, CP4EPSPS and PAT are not likely to be allergenic. In addition, there is no report of allergenicity of any Cry protein from *B. thurengiensis*, and most of them have already been accepted by EFSA for inclusion into various GMO species to control insect pest. This may indicate that Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1 and Cry34/35 Ab1 are not likely to be allergenic.
However, it must be emphasized that Cry1A.105 displays high aminoacid sequence identity with Cry1Ac and that Cry1Ac has been proposed as an adjuvant for vaccines (Vasquez et al, 1999, Vasquez-Padron et al. 1999, Moreno-Fieros et al. 2003, Esquivel-Perez et al. 2005), which means that this protein is able to enhance the immune responses against antigens that are co-administered, which is not uncommon for a bacterial protein. Other proteins of the Cry family are also suspected of showing adjuvant properties (Calderon et al. 2007). Therefore, doubt may arise about Cry2Ab2, Cry1F, Cry3Bb1 and Cry34/35 Ab1. The consequence of the presence of such immuno-stimulant in a plant destined to human consumption is not known. Particularly the adjuvant effect via intestinal route is poorly documented. The single concentration of Cry1A.105 in maize grains is compatible with the possibility of an adjuvant effect in the context of normal maize grain consumption (but the

concentration after processing of the maize or after cooking is not known). If all Cry proteins also have such adjuvant capacity, the adjuvant effect may be multiplied in MON89034x1507xMON88017x59122 maize. It is not known whether the presence of these Cry proteins in maize may elicit sensitization against the other maize proteins upon ingestion (and which type of sensitization?).
This point needs to be clarified. Therefore, it is relevant to at least study in mice the immune responses against maize proteins when the animals are fed MON89034x1507xMON88017x59122 maize.

Assessment of the allergenicity of the whole GM plant or crop.
The applicant did not assess the allergenicity of the whole GM plant. Care should be taken not to underestimate maize food allergy. Indeed, some maize allergens have been described in the literature (Pasini et al. 2002, Pastorello et al. 2003, Weichel et al. 2006, Fasoli et al. 2009) and, recently, patients showed maize-induced anaphylaxis in double-blind placebo-controlled food challenge, with reactions to as little as 100 mg of maize (Scibilia et al. 2008). This reinforces the need to evaluate the allergenicity of the whole GM plant, as care must be taken that no increase in maize allergy incidence appears due to excessive allergen expression levels in modified maize.
It is relevant to analyze whether the expression levels of known maize allergens is increased in the genetically modified maize grains or to analyze whether the overall allergenicity of the modified maize has increased, as compared to a natural counterpart. This is relevant as, theoretically, the introduction of all these new traits, through multiple cascade interactions, might have modified the expression level of some endogenous maize proteins. Patient IgE binding to modified maize grain extract or titration of known major allergens of maize should be carried out.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1

A broiler chicken feeding experiment with grain of the MON 89034x1507xMON 88017x59122 was performed. No negative effects were noted.

Comment 2

P.67 & 69 (Table 20) of the Technical Dossier: it is a pity that ADL is not included as fibre parameter.

It is a pity that the nutritive value of MON 89034 × 1507 × MON 88017 × 59122 maize for different animals species (poultry, pigs and ruminants) is not reported, or the in vitro digestibility, as a parameter to indicate its nutritional equivalence.

A feeding experiment with broilers (Davis, 2008), lasting 42 days, including ±60% maize in the starting period and more than 60% in the finishing period, showed no negative effects on growth rate feed utilisation, mortality and overall carcass yield and quality.

Comment 3

The information provided in the application is sufficient.

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

Comments/Questions of the expert(s)

Comment 1

No questions.

Comment 2

The information provided in the application is sufficient.

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

NOT APPLICABLE

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 information provided in the application is sufficient. (The scope of the application does not include authorization for the cultivation of 98140 maize seed products in the EU).

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient. (The scope of the application does not include authorization for the cultivation of 98140 maize seed products in the EU).

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

Comment 1

This topic is not of relevance, as the application of MON 89034 × 1507 × MON 88017 × 59122 maize does not intend to cultivate the maize crop.

Comment 2

The information provided in the application is sufficient. (The scope of the application does not include authorization for the cultivation of 98140 maize seed products in the EU).

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient. (The scope of the application does not include authorization for the cultivation of 98140 maize seed products in the EU).

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

Comments/Questions of the expert(s)

D.9.6 Effects on human health

Comments/Questions of the expert(s)

Comment 1

No questions.

Comment 2

Based on the effect of MON 89034 × 1507 × MON 88017 × 59122 maize on the mortality rate in broilers, no short term harmful effects are expected in humans.

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1

No questions.

Comment 2

The feeding experiment reported by Davis (2008) did not show negative effects of MON 89034 × 1507 × MON 88017 × 59122 maize on the mortality rate in broilers, fed with 60% or even slightly more in their diet during 42 days.

Comment 3

One study was found concerning the performance of broilers. The reported data were on pen level so that 10 replications, i.e. 5 pens per sex, were available. Based on the reported variability within treatments, the statistical power is not sufficient to find significant differences. Nevertheless based on the interpretation of the reported intervals of confidence, statistical significant differences were reported, but were not mentioned in the conclusions, because these were not considered as biological significant. However, it is worthwhile to mention that the SEM of some parameters was on average 3 times larger in the reported treatment groups than in the control, and that mortality rate was rather high. The calculation of the feed conversion ratio was not as exact as being possible.

Comment 4

The information provided in the application is sufficient.

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

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

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient. (The scope of the application does not include authorization for the cultivation of 98140 maize seed products in the EU).

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

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

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

References

Bannon, G., Fu, T.J., Kimber, I., Hinton, D.M. (2003). Protein digestibility and relevance to allergenicity. Environ. Health Perspect. 111: 1122-1124.

Bernstein JA, Bernstein IL, Bucchini L, Goldman LR, Hamilton RG, Lehrer S, Rubin C, Sampson HA. (2003) Clinical and laboratory investigation of allergy to genetically modified foods. Environ Health Perspect. 111(8):1114-21.

Buchmann CA, Nersesyan A, Kopp B, et al. (2007) Dihydroxi-7-methoxy-1,4-bezoxazin-3-one (DIMBOA) and 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA), two naturally occurring benzoxazinones contained in sprouts of Gramineae are potent aneugens in human-derived liver cells (HepG2). Cancer Letters 246: 290-299.

Calderón et al. (2007) Adjuvant effects of crystal proteins from a Mexican strain of Bacillus thuringiensis on the mouse humoral response. Biologicals, 35 :271-6.

Ebo DG, Hagendorens MM, Bridts CH, Schuerwegh AJ, De Clerck LS, Stevens WJ. (2005) Flow cytometric analysis of in vitro activated basophils, specific IgE and skin tests in the diagnosis of pollen-associated food allergy. Cytometry B Clin Cytom. 64(1):28-33.

Ebo DG, Stevens WJ. (2001) IgE-mediated food allergy--extensive review of the literature. Acta Clin Belg. 56(4):234-47.

Esquivel-Pérez et al. (2005) Mucosal and systemic adjuvant effects of cholera toxin and Cry1Ac protoxin on the specific antibody response to HIV-1 C4/V3 peptides are different and depend on the antigen co-administered. Viral Immunol, 18:695-708

Fasoli et al. (2009) Searching for allergens in maize kernels via proteomic tools. J Proteomics, 72 :501-10

FeronVJ, Til HP, Devrijer F et al. (1991) Aldehydes- occurence, carcinogenic potential mechanisms of action and risk assesment. Mut Res 259:363-385.

Helm RM. (2003) Food biotechnology: is this good or bad? Implications to allergic diseases. Ann Allergy Asthma Immunol. 90(6 Suppl 3):90-8.

Herman, R.A., Storer, N.P., Gao, Y. (2006). Digestion assays in allergenicity assessment of transgenic proteins. Environ. Health Perspect. 114: 1154-1157.

Hippen, A.R., Linke, K.N., Kalscheur, K.F., Schingoethe, D.J., Garcia, A.D. (2003). Increased concentration of wet corn distillers grains in dairy cow diets. J. Dairy Sci. 86 (Suppl. 1): 340 (Abstr.).

Janicek, B.N., Kononoff P.J., Gehman, A.M., Doane, P.H. (2008). The effect of feeding dried distillers grains plus solubles on milk production and excretion of urinary purine derivatives. J. Dairy Sci. 91: 3544-3553.

Kalscheur, K.L., A.L. Justin, A.L. Hippen, and D.J. Schingoethe (2004). Increasing wet distillers grains in the diets of dairy cows on milk production and nutrient utilization. J. Dairy Sci. 87 (Supp. 1): 465-466. (Abstr.).

Klun JA, Robinson JF (1969) Concentration of two 1,4 benzoxazinones in dent corn of various stages of development of the plant and its relation to resistance of the host plant to the European corn borer J Econ Entomol 62: 214-220.

Moreno-Fiéros et al. (2003) Intranasal Cry1Ac protoxin is an effective mucosal and systemic carrier and adjuvant of Streptococcus pneumoniae polysaccharides in mice. Scand J Immunol 2003,57:45-55

Ouellet, D.R., Lapierre, H., Chiquette, J. (2003). Effects of corn silage processing and amino acid supplementation on the Performance of lactating dairy cows. J. Dairy Sci. 86: 3675-3684

Pasini et al. (2002) IgE-mediated allergy to corn: a 50 kDa protein, belonging to the Reduced Soluble Proteins, is a major allergen. *Allergy*, 57:98-106

Pastorello et al. (2003) Lipid-transfer protein is the major maize allergen maintaining IgE-binding activity after cooking at 100 degrees C, as demonstrated in anaphylactic patients and patients with positive double-blind, placebo-controlled food challenge results. *J Allergy Clin Immunol,* 112;775-83

Roeber, D.L., Gill, R.K., DiCostanzo, A. (2005). Meat quality responses to feeding distiller's grains to finishing Holstein steers. J. Anim. Sci. 83: 2455–2460.

Scibilia J, Pastorello EA, Zisa G, Ottolenghi A, Ballmer-Weber B, Pravettoni V, Scovena E, Robino A, Ortolani C. (2008) Maize food allergy: a double-blind placebo-controlled study. *Clin Exp Allergy*, 38:1943-9

Séralini, G.E., Cellier, D., Spiroux de Vendomois, J. (2007). New analysis of a rat feeding study with a genetically modified maize reveals signs of hepatorenal toxicity. Arch. Environ. Contam. Toxicol. 52: 596-602.

Shulmina AI, Dronova LA, Shubin VV, et al. **(**1985) Determination of the secondary structure of the chymotrypsin inhibitor from corn by the circular-dichroism method**.** BIOCHEMISTRY-MOSCOW 50**,** 7**:** 980-982

Spök, A., Gaugitsch, H., Laffer, S., Pauli, G., Saito, H., Sampson, H., Sibanda, E., Thomas, W., van Hage, W., Valenta, R. (2005). Suggestions for the assessment of the allergenic potential of genetically modified organisms. Int. Arch. Allergy Immunol. 137:167-180.

Vásquez et al. (1999) Bacillus thuringiensis Cry1Ac Protoxin is a Potent Systemic and Mucosal Adjuvant.Scand J Immunol, 49:578-84

Vásquez-Padrón et al. (1999) Intragastric and intraperitoneal administration of Cry1Ac protoxin from Bacillus thuringiensis induces systemic and mucosal antibody responses in mice. Life Sci 1999, 64:1897-912

Weichel et al. (2006) Screening the allergenic repertoires of wheat and maize with sera from double-blind, placebo-controlled food challenge positive patients. *Allergy,* 61:128-35.

Bioveiligheidsraad
Conseil de Biosécurité

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Secretariaat
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Application EFSA/GMO/CZ/2008/62
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 17 March 2009
Coordinator: Prof. dr. ir. Dirk Reheul
Experts: Pascal Cadot (Consultant), Armand Christophe (UGent), Leo Fiems (ILVO), Rony Geers (KUL), André Huyghebaert (UGent), Peter Smet (Consultant), Wim Stevens (UA), Jan Van Doorsselaere (KH Zuid-West Vlaanderen), Hadewijch Vanhooren (KUL), Michel Van Koninckxloo (HEPHO)
Domains of expertise of experts involved: Genetics, molecular characterisation, human nutrition, animal nutrition, analysis food/feed, traceability of alimentary chain, substantial equivalence, toxicology in vitro, in vivo, general biochemistry, immunology, alimentary allergology, agronomy, agro-ecology, ecotoxicology, herbicide tolerance
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

INTRODUCTION

Dossier **EFSA/GMO/CZ/2008/62** concerns an application of the company **Monsanto & Dow AgroSciences** for the marketing of the genetically modified **maize MON89034 x 1507 x MON88017 x 59122** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 3 March 2009.
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)

Comments posted on the EFSAnet

The comments are structured according to 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). The comments below are those that were posted on the EFSAnet. 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. The compilation of all the comments that were received from the experts (including the references) is given in a separate document (ref. BAC_2009_749).

GENERAL COMMENTS

Comments/Questions of the expert(s)

Serious problems are not really expected by the application of MON89034 x 1507 x MON88017 x 59122 maize. The safety aspects of the multiple challenge, due to the combination of the newly inserted proteins, are rather weakly demonstrated.

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

It is important to note that the safety of MON 89034 and MON 88017 is still under scientific review by the EFSA GMO panel (Technical dossier I, page 14).

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

No comments

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

P31; could the authors state why this construct also contains –what it seems- partial cry1F and pat fragments?
What is the size is of these fragments and why is hybridisation not seen in Southern blots?

D. INFORMATION RELATING TO THE GM PLANT

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

No comments

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

Comments/Questions of the expert(s)

P36: is it possible to add data on the chromosome location of the four inserts (and segregation analysis in relation with the breeding scheme in fig 13 p51)?

P39: what can be said about the band of 4.2 Kb? Is this due to partial restriction of genomic DNA?

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

In part 1 of the technical dossier we can read p.45 “For the PAT protein, expression was higher in the combined trait product as compared to 1507 and 59122” but the Table 12 (PAT) shows that the values for PAT protein levels in grain collected from MON 89034 × 1507 × MON 88017 × 59122 (0.050 µg/g dw) are similar to those of 59122 (0.049). Is there no contradiction between the statement “… the levels of Cry1A.105….are comparable to the protein levels in the positive controls…” (Technical dossier, part I, page 45) and the data provided in Table 6 for this protein (4.3 vs 2.8 in the control)? (almost no overlap in range; means are about 3 SD different).
P45 we are not sure whether statistically there is a difference in Pat levels between 59122 and (89034 x 1507 x 88017 x 59122) as stated and therefore to our opinion the sentence: "This is likely due to the presence of multiple copies of the pat gene…" should be deleted.

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

No comments

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

No comments

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

No comments

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)

Table 21, Technical dossier, part I, page 73. Is there no mistake in the reported mean value for linoleic acid in the control grain? This value seems unlikely to me (for a non high-oleic acid corn oil).

The presence of trypsin and chymotrypsin inhibitors have been described in corn (Shulima et al., 1985). No data are reported on the level of these antinutrients.

Palmitoleic acid is not mentioned in table 20 of the Technical Dossier, part I, page 69. Yet it is known to be a minor component in corn oil and was found in some positive controls. The claim N/A (not available) is unlikely as the fatty acid composition was determined by gas chromatography. This omission is of no nutritional importance however.

It is not clear what is meant with “maize tissues” in the second paragraph on page 65 of Technical Dossier, part I. (…maize tissues that are consumed??) Printing error?

D.7.2 Production of material for comparative assessment

No comments

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

The selection of the compounds for analysis is according to the OECD consensus document 6 (2002). However, considering that DIMBOA and its glycoside may total 1% of dry weight in conventional corn plants (Klun et al.,1969) and that mutagenic effects in human cell lines have recently been demonstrated (Buchmann et al., 2007), it seems of value to determine these components in corn containing stacked events. Hormonal effects of its degradation product MBOA have been described in rodents (in OECD report 6, page 28).

It is claimed that 2-furaldehyde was determined (Technical Dossier I, page 82). Yet, I have found no data on levels of this component. Did I miss them or were they not reported? Although furfural has GRAS status, it has been suggested that an increase of the furfural level in food stuff should be avoided and that furfural is considered as a dietary risk factor for cancer (Feron et al.,1991).

Grain and forage are analyzed for nutrients, according to the OECD documents. In addition other constituents with growing importance are included in the study.
On the other hand a rather traditional approach was chosen for the proximate nutrients. No information is available on the composition of the carbohydrate fraction as it is calculated “by

difference". This is regrettable from a nutritional point of view as more and more attention is given to the type of carbohydrates present in human food.
The same remark further applies for the fibre fraction, as mentioned several times before in previous dossiers.
The OECD document needs to be adapted to current knowledge in human nutrition (see also EU definition of fibre).

D.7.4 Agronomic traits

No comments

D.7.5 Product specification

No comments

D.7.6 Effect of processing

No comments

D.7.7 Anticipated intake/extent of use

No comments

D.7.8 Toxicology

Comments/Questions of the expert(s)

In case of MON 89034 × 1507 × MON 88017 × 59122 there may be a multiple challenge, which can be more harmful than any individual newly inserted proteins. It is highly desirable to refer to studies that have demonstrated that the combination of all these newly inserted proteins is not detrimental. However, the modes and sites of biological activity are different for the Cry, PAT and CP4 EPSPS proteins and there is no known or conceivable mechanism of interaction between these proteins which could lead to adverse health effects in animals or humans. Does this observation really guarantee full safety ?

A study of Séralini et al. (2007) revealed signs of hepatorenal toxicity in rats due to the genetically modified Maize MON 863, which contains a variant of the Bacillus
thuringiensis Cry3Bb1 gene. MON 89034 × 1507 × MON 88017 × 59122 produces Cry3Bb1 insecticidal protein and may therefore be similar to MON 863.

According to the Ouellet et al. (2003) the TMR contained 0.446 maize silage (containing approximately 70 g protein/kg dry matter; DM) and 0.188 cracked maize (containing approximately 100 g protein/kg DM), resulting in 40 g maize protein/kg dietary DM. However, DDGS (containing approximately 300 g

protein/kg DM), the by-product from the bioethanol production is sometimes incorporated in diets for **dairy cows** at 0.3 - 0.4 (Hippen et al, 2003; Kalscheur et al., 2004; Janicek et al., 2008), and distillers grains were be used at 50% in diets for finishing beef cattle (Roeber et al., 2005). Diets with 40% DDGS may yield ±120 g maize protein/kg dietary DM, which means that:

- the protein amount coming from maize, via DDGS, is 3 x higher than the protein amount coming from directly from MON 89034 × 1507 × MON 88017 × 59122 grain and/or silage
- this protein also contain about 3 x more newly inserted protein

What is the effect of such a diet on the safety for the animals? However, the animal feeding study, reported by Davis (2008) and conducted with relatively high incorporation levels in the diets of broilers, did not show health problems which can be attributed to this MON 89034 × 1507 × MON 88017 × 59122 maize, which is an indication of a safe use in **monogastric animals.**

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

The only question we have concerning this item is whether an up-to-date sequence homology search has been performed for each of the proteins?

D.7.8.2 Testing of new constituents other than proteins

No comments

D.7.8.4 Testing of the whole GM food/feed

No comments

D.7.9 Allergenicity

Comments/Questions of the expert(s)

1) Assessment of the allergenicity of the newly expressed proteins.

It must be emphasized that Cry1A.105 displays high aminoacid sequence identity with Cry1Ac and that Cry1Ac has been proposed as an adjuvant for vaccines (Vasquez et al, 1999, Vasquez-Padron et al. 1999, Moreno-Fieros et al. 2003, Esquivel-Perez et al. 2005), which means that this protein is able to enhance the immune responses against antigens that are co-administered, which is not uncommon for a bacterial protein. Other proteins of the Cry family are also suspected of showing adjuvant properties (Calderon et al. 2007). Therefore, doubt may arise about Cry2Ab2, Cry1F, Cry3Bb1 and Cry34/35 Ab1. The consequence of the presence of such immuno-stimulant in a plant destined to human consumption is not known. Particularly the adjuvant effect via intestinal route is poorly documented. The single concentration of Cry1A.105 in maize grains is compatible with the possibility of an adjuvant effect in the context of normal maize grain consumption (but the concentration after processing of the maize or after cooking is not known). If all Cry proteins also have such adjuvant capacity, the adjuvant effect may be multiplied in MON89034x1507xMON88017x59122 maize. It is not

known whether the presence of these Cry proteins in maize may elicit sensitization against the other maize proteins upon ingestion (and which type of sensitization?).
This point needs to be clarified. Therefore, it is relevant to at least study in mice the immune responses against maize proteins when the animals are fed MON89034x1507xMON88017x59122 maize.

2) Assessment of the allergenicity of the whole GM plant or crop.
The applicant did not assess the allergenicity of the whole GM plant. Care should be taken not to underestimate maize food allergy. Indeed, some maize allergens have been described in the literature (Pasini et al. 2002, Pastorello et al. 2003, Weichel et al. 2006, Fasoli et al. 2009) and, recently, patients showed maize-induced anaphylaxis in double-blind placebo-controlled food challenge, with reactions to as little as 100 mg of maize (Scibilia et al. 2008). This reinforces the need to evaluate the allergenicity of the whole GM plant, as care must be taken that no increase in maize allergy incidence appears due to excessive allergen expression levels in modified maize.
It is relevant to analyze whether the expression levels of known maize allergens is increased in the genetically modified maize grains or to analyze whether the overall allergenicity of the modified maize has increased, as compared to a natural counterpart. This is relevant as, theoretically, the introduction of all these new traits, through multiple cascade interactions, might have modified the expression level of some endogenous maize proteins. Patient IgE binding to modified maize grain extract or titration of known major allergens of maize should be carried out.
The classical evaluation methods have been used and do not demonstrate the GMO to be a product which might be associated with allergy development. However, since the methods used are not completely predictive for allergy development long term follow up is warranted, e.g. the rapid digestibility in simulated digestive fluids is not a guarantee for safety. Bannon et al. (2003) and Herman et al. (2006) concluded that the use of the SGF technique to predict the allergenic status of the proteins remains uncertain and Spök et al (2005) have shown that digestibility studies can not be considered as suitable tools to address the allergenic potential of a protein.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

A broiler chicken feeding experiment with grain of the MON 89034x1507xMON 88017x59122 was performed. No negative effects were noted.
The reported data were on pen level so that 10 replications, i.e. 5 pens per sex, were available. Based on the reported variability within treatments, the statistical power is not sufficient to find significant differences. Nevertheless based on the interpretation of the reported intervals of confidence, statistical significant differences were reported, but were not mentioned in the conclusions, because these were not considered as biological significant. However, it is worthwhile to mention that the SEM of some parameters was on average 3 times larger in the reported treatment groups than in the control, and that mortality rate was rather high. The calculation of the feed conversion ratio was not as exact as being possible.

P.67 & 69 (Table 20) of the Technical Dossier: it is a pity that ADL is not included as fibre parameter.

It is a pity that the nutritive value of MON 89034 × 1507 × MON 88017 × 59122 maize for different animals species (poultry, pigs and ruminants) is not reported, or the in vitro digestibility, as a parameter to indicate its nutritional equivalence.

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

No comments

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

NOT APPLICABLE

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

No comments

D.9.2 Selective advantage or disadvantage

No comments

D.9.3 Potential for gene transfer

No comments

D.9.4 Interactions between the GM plant and target organism

No comments

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

No comments

D.9.6 Effects on human health

No comments

D.9.7 Effects on animal health

No comments

D.9.8 Effects on biogeochemical processes

No comments

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

No comments

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

No comments

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

No comments

D.11.2 Interplay between environmental risk assessment and monitoring

No comments

D.11.3 Case-specific GM plant monitoring

No comments

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

No comments

D.11.5 Reporting the results of monitoring

No comments

References

see document BAC_2009_949 in annex

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Conseil de Biosécurité

21 September 2010

Secretariaat
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O./ref.: WIV-ISP/41/BAC/2010_0902bis

Inaugural meeting of the EFSA scientific Network for Risk Assessment of GMOs

22-23 November 2010, Parma, Italy

List of issues on risk assessment of GMOs, sharing best practice, etc. that the Biosafety Advisory Council and/or the SBB would like to propose for discussion at the first and subsequent meetings of the EFSA Scientific Network
(notably on the basis of questions already submitted to EFSA by the BAC on 26/06/2008 - ref doc WIV-ISP/BAC/2008_777)

(1) General issues

- It would be interesting to find a more appropriate way to share information with Member States (MS) when additional info is provided by the applicant.

- In case of submission of related dossiers, it would be interesting to find a way to communicate to the MS what documents are new or updated in the new dossier.

(2) Specific issues related to Food & Feed safety assessment and/or molecular characterisation

2.1. OECD-related issues:

In the dossiers, the proximate analysis is performed according to the recommendations of the OECD, which means that only the crude fibre method is applied. This conventional approach is indeed suitable for animal feed but that, in the case of foods, the dietary fibre method (with a specification as soluble and insoluble fibre) should be applied. This point is under discussion in the Task Force of the OECD. A more general question is: shall we wait for the publication of the OECD consensus documents to adapt the requirements for data to be submitted in EFSA applications?

Maize is known to contain a lot of hydroxamic acids and derivative compounds (Cambier *et al*., 1999)[1]. These aromatic compounds are potentially toxic and play a major role in maize resistance against insect and diseases. A recent paper of Nie *et al.* (2005)[2] investigated the

[1] Cambier, V., Hance, T. and de Hoffmann, E. (1999) Non-injured Maize contains several 1,4-Benzoaxzin-3-one related compounds but only as glucoconjugates. Phytochemical analysis, 10: 119-126.
[2] Nie, C.R., Luo S.M., Lin, C.X., Zeng, R.S., Huang, J.H., Wang, J.W. (2005). Status of DIMBOA and phenolic acids in transgenic *Bt* corn. Australian Journal of Agricultural Research 8: 833-837

status of DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) and phenolic acids in leaves of some transgenic *Bt* corn hybrids. They showed that the introduction of the *Bt* gene could have adverse effects on the biosynthesis and accumulation of DIMBOA and some phenolic acids, such as ferulic acid, in the corn plants. We want to discuss why DIMBOA is not considered in the compositional analysis as it is now commonly used as an indicator of resistance by maize breeders, but can also influence food and feed toxicity and allergenicity.

2.2. Other issues

- In several dossiers, some animal tests were not designed according to scientific standards and should have included more animals per treatment to increase the power of the statistical analysis or the sensitivity of the trial. In consequence, it was not possible to draw any scientific conclusions from those trials. All scientific experiments presented in the dossiers (including supplementary studies not formally required on the basis of the EFSA guidance) should comply with standard of good design and quality for appropriate statistical analysis of the data, and should be fully considered in the context of the overall risk assessment. It would be interesting to know the views of other experts on how they deal with poorly designed experiments that are not strictly required (e.g. according to the guidelines of EFSA) to come to a conclusion on risk.

- In the updated guidance document for the risk assessment of GM plants and derived food & feed[3], the risk/safety assessment is based as before on the principle of substantial equivalence. We would like to discuss to what extent and on the basis of what scientific reasoning and what present knowledge the molecular characterisation combined with the compositional analysis, could provide enough information relevant for the risk/safety assessment to rule out any potential unintended effects and to allow the applicants not doing toxicity tests *in vivo*?

- It would be interesting to discuss to what extent bioinformatics can provide information on the potential allergenicity of GMOs.

(3) Specific issues related to the Environmental Risk Assessment (ERA) of GMOs

- It would be interesting to discuss procedural aspects related to

a) delegation of an ERA to MS, in particular of applications with stacked events,
b) validation check of cultivation dossiers: what is exactly checked by EFSA?
c) MS comments: how are they dealt with by MS involved in the evaluation of ERAs and later on by EFSA?

- We would like to know the view on MS on IRM: do they consider this issue as an environmental or rather an agronomic issue?

[3] The EFSA Journal (2008) 727, 1-135

Issues not retained by the Biosafety Council but to possibly bring back further for the discussion within the EFSA scientific Network for Risk Assessment of GMOs.

- In relation to potential impacts of agricultural practices associated to GM crops, it would be interesting to :
  - a) discuss and clarify better the question of selection of receiving environments (including the different zoning concepts, casually in combination with the main cultivation areas of the crops, on a case/case basis) and of assessment endpoints (final protection goals) in order to provide more guidance and precise requirements to the applicants on these concerns.
  - b) discuss more deeply the problem of defining a baseline in a dynamic environment with evolving agricultural systems. As such, it would be interesting to discuss the possibility of requiring different comparative cultivation systems following the receiving environment and different comparative cultivation systems in each receiving environment (to be defined following those in practice in the different receiving environments). This would maybe better allow the final decision of assessors and deciders to be a reasoned choice rather than a yes/no answer based on one single dubious comparator.
  - c) discuss the rational of imposing ecotoxicological tests only during 2 consecutive years on 3 different sites.
  - d) discuss the question of the probability of horizontal gene transfer to microorganisms: it seems to us that this should be tested under natural conditions of different receiving environments - resulting casually from different agricultural practices - before being able to be considered as a " rare event " . [4]

- Presently, the post-market monitoring is accomplished exclusively by persons coming in contact with GMOs through their current professional activities (traders, producers of feed supplies, …..) . Whereas the information brought by those people is surely useful, is it relevant that the whole process of monitoring relies only on their observations and reports? Should the monitoring process not be primarily in the hands of professionals of monitoring systems and of the elements to be observed? Is it not contestable, in the context of a legislation based on the precautionary principle, to allow the placing on the market of GMOs for which scientific uncertainties on potential adverse effects are often proposed to be followed in the post-marketing monitoring phase without having a more professional monitoring system? We would strongly like reassuring information on how and when such a system will be functioning at the level of the EU? A study was made in Belgium already some years ago, with the conclusion that no existing monitoring system in our country could adequately realize the monitoring of adverse effects on health of GMOs put on the market. We are thus waiting for a guidance of a monitoring system coordinated at the EU level before taking any initiative at the national level. On the basis of the present market of GMOs import in the EU, monitoring animal health (implication of veterinarians) would in any case be a least as important as monitoring human health. One puzzling question interesting to discuss in the functioning of such a monitoring system would be - supposing for ex. that such a relationship would exist - the establishment of cause-effect relationship between a slowly appearing chronic disease and the consumption of a variety of GMOs, taking into account the rather short lifetime of agronomic varieties succeeding each other on the market. Whereas being beyond EFSA mandate, the question of the financing of a monitoring system is also an important point to discuss and to take into account.

4 At the meeting of 17 September the member of the BAC asked to transmit this issue under point d) to EFSA. SBB thought it twas more appropriate to wait for the new EFSA guidelines on environmental risk assessement