Bioveiligheidsraad
Conseil de Biosécurité

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Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2012_0785

Title: Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/DE/2010/82 from Syngenta under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/DE/2010/82 was submitted by Syngenta on 23 July 2010 for the marketing of genetically modified maize MIR162 for food and feed uses, import and processing within the framework of Regulation (EC) No. 1829/2003[1]. Maize MIR162 expresses the gene of the Vip3Aa20 protein that confers resistance against specific lepidopteran insects and the gene of phosphomannose isomerase (PMI) serving as selection marker.

The application contains a lot of information that is similar to the earlier submitted dossiers EFSA/GMO/DE/2009/66 (Bt11 x MIR162 x MIR604 x GA21) and EFSA/GMO/DE/2009/67 (Bt11 x MIR162 x GA21). It was officially acknowledged by EFSA on 24 August 2010. 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 being part of the products).

Within the framework of this consultation, the Belgian Biosafety Advisory Council (BAC), under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier, chosen from the common list of experts drawn up by the BAC and the Biosafety and Biotechnology Unit (SBB). Two experts accepted to evaluate the parts of the dossier that had not been evaluated in the frame of applications 66 and 67. They formulated a number of comments to the dossier, which were edited by the coordinator and added to the comments of the six experts who evaluated applications 66 and 67. See Annex I for an overview of all the comments and for the list of comments actually placed on the EFSAnet on 29 November 2010.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 31 May 2012 (EFSA Journal 2012; 10(6):2756[2], and published together with the responses from the EFSA GMO Panel to comments submitted by the experts during the three-month consultation period.

On 25 June 2012 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, complementary information regarding toxicity sent by the company to EFSA in February 2012 was provided to the coordinator and the experts who evaluated this aspect of the application. The comments

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed (OJ L 268, 18.10.2003, p.1).
[2] See http://www.efsa.europa.eu/en/efsajournal/pub/2756.htm

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

Scientific evaluation

1. Environmental risk assessment

According to the Biosafety Advisory Council no major risks were identified concerning the environment[3].

2. Molecular characterisation

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

3. Assessment of food/feed safety and nutritional value

3.1. Assessment of compositional analysis

The compositional analysis follows the OECD recommendations. However, the Biosafety Advisory Council considers that, although not required by the OECD, 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 document accordingly.

3.2. Assessment of toxicity

The Biosafety Advisory Council is of the opinion that the dossier takes away major safety concerns, but it leaves a minor concern based on statistical principles. Hence the BAC proposes a tough monitoring on this aspect.

With regard to the subchronic oral toxicity of the Vip3Aa20 protein present in MIR162 maize, the data provided in the application were not satisfactory. But on request of EFSA the applicant provided results of a new 28 days oral toxicity study in rats. These data have been reviewed by the Belgian experts: no adverse effects were observed at the highest dose tested which is much higher than the expected daily uptake by humans.

Although the animal trials were properly designed, from a statistical point of view, the ex post analysis of the 28 days oral toxicity study and of the previously submitted 90-day rat feeding study with grain of maize MIR162, reveals that the number of replications was too small to detect statistically significant differences, if present.

Concerning the PMI protein present in MIR162 maize, the Biosafety Council considers that the data provided by the applicant adequately demonstrates the absence of potential toxicity.

[3] 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.

3.3. Assessment of allergenicity

The potential allergenicity of the newly expressed proteins has been assessed. The Biosafety Council considers Vip3Aa20 protein as unlikely to be allergenic. The BAC is however concerned about the potential allergenicity of the PMI protein, as was already mentioned in previous advices on GM maize expressing the same marker gene. The BAC sticks to its former assessments given for applications EFSA/GMO/UK/2007/48, EFSA/GMO/UK/2007/50 and EFSA/GMO/UK/2008/56[4].

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. However from a statistical point of view the ex post analysis revealed that in the feeding trial with broilers the number of replications was too to detect a statistically significant difference, if present.

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.
As the statistical power of the animal trials was not perfect, the Biosafety Advisory Council advises to be vigilant on the chronic toxicity and nutritional value.

[4] Ref. BAC/2010/0952, BAC/2010/956, BAC/2010/058 available on the internet site < http://www.bio-council.be/bac_advices.html >

Conclusion

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

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

A minority of the members of the BAC agrees with the GMO panel of EFSA when it says that the maize MIR162 is unlikely to have an adverse effect on human health in the context of its intended uses. A majority disagrees, since identified potential allergenicity of the transgene PMI protein has not been appropriately tested with *in vitro* and/or *in vivo* tests.
Therefore the BAC advises a conditional approval provided a tough monitoring on human health is conducted.

p.o. Dr. Ph. Herman

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

Annex I: Full comments of experts in charge of evaluating application EFSA/GMO/DE/2010/82 and comments submitted on the EFSAnet (ref. BAC_2010_1105)

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29-11-2010

Secretariaat
Secrétariat

N./réf. : WIV-ISP/41/BAC_2010_1105
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Compilation of comments of experts in charge of evaluating the application EFSA/GMO/DE/2010/82
and
Comments submitted on the EFSAnet on mandate of the Biosafety Council

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 17 September 2010
Coordinator: Prof. Dirk Reheul
Experts for AP 66 and 67: Pascal Cadot (Consultant), Rony Geers (KUL), André Huyghebaert (UGent), Peter Smet (Consultant), Jan Van Doorsselaere (KH Zuid-West Vlaanderen), Hadewijch Vanhooren (KUL)
Experts who evaluated the additional data submitted for AP 82: Peter Smet (Consultant), Jan Van Doorsselaere (KH Zuid-West Vlaanderen)
Domains of expertise of experts involved: Genetics, molecular characterisation, human nutrition, animal nutrition, traceability of alimentary chain, analysis food/feed, substantial equivalence, toxicology in vitro and in vivo, general biochemistry, immunology, alimentary allergology, ecology, ecotoxicology, population genetics, plant-insect relations, nature conservation, herbicide tolerance.
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman, Katia Pauwels

INTRODUCTION

Dossier **EFSA/GMO/DE/2010/82** concerns an application of the company **Syngenta** for the renewal of the marketing authorisation of the genetically modified **maize MIR162** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 24 August 2010.

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)

This dossier **EFSA/GMO/DE/2010/82** contains a lot of information that is similar to the recent applications **EFSA/GMO/DE/2009/66 (Bt11 x MIR162 x MIR604 x GA21)** and **EFSA/GMO/DE/2009/67 (Bt11 x MIR162 x GA21)**. In order not to duplicate any efforts the experts of the Biosafety Advisory Council were asked to evaluate the parts of the dossier on **MIR162** that have not been evaluated before. These are:
- regarding molecular characterisation: MIR162 event updated bioinformatic search, MIR162 event protein expression data, presentation of protein expression data;
- regarding compositional analysis and toxicity: compositional characteristics of MIR162 maize and its comparator(s), statistical analysis of additional components, statistical analysis of the comparison between genetically modified maize and its control, for all parameters in each location, previous exposure of humans and animals to *B. thuringiensis* VIP3a proteins, oral toxicity of VIP3aa20, simulated gastric fluid assay with VIP3aa20, equivalence study for vip3aa20.
It only concerns parts **D2, D.3, D.7.1, D.7.4, D.7.8**. and **D.7.8.1**.. below.

As application EFSA/GMO/DE/2010/82 will be handled by EFSA separately the document repeats the comments relevant for MIR162 received from the experts for applications EFSA/GMO/DE/2009/66 and EFSA/GMO/DE/2009/67. Depending on their expertise, the experts who evaluated these applications were asked to evaluate the genetically modified plant considered in the application on its 1) molecular, 2) environmental, 3) allergenicity, 4) toxicity and/or 5) food and feed aspects. It was expected that the expert should evaluate if the information provided in the application is sufficient in order to state that the marketing of the genetically modified plant for its intended uses, will not raise any problems for the environment or human or animal health. If information is lacking, the expert was asked to indicate which information should be provided and what the scientifically reasoning is behind this demand.
The comments are structured as in the "Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed" (EFSA Journal (2004), 99, 1-94). Items are left blank when no comments have been received either because the expert(s) focused on other related aspects, or because for this dossier the panel of experts who accepted to evaluate the dossier didn't have the needed expertise to review this part of the dossier.
It should be noted that all the comments received from the experts are considered in the evaluation of this dossier and in formulating the final advice of the Biosafety Advisory Council. Comments placed on the EFSAnet are indicated in grey.

List of comments received from the experts

GENERAL COMMENTS

Comments/Questions of the expert(s)

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1 (as given for applications EFSA/GMO/DE/2009/66 & 67)

No comments

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

Comments/Questions of the expert(s)

Comment 1 (as given for applications EFSA/GMO/DE/2009/66 & 67)

No comments

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Comment 1

Reviewer agrees with the conclusions from appendix 16 in Appendix 36 of the application (Molecular characterization of event MIR162):
"Data from Southern analyses and DNA sequencing demonstrated that single copies of the *vip3Aa20* gene and *pmi* gene are present in Event MIR162 maize. Additionally, Event MIR162 maize does not contain any of the backbone sequences from the transformation plasmid pNOV1300. Sequence analysis revealed two single nucleotide changes within the coding sequence of *vip3Aa20*. Only one of the nucleotide changes encoded an amino acid change, where methionine at position 129 has been substituted by isoleucine. Statistical analysis over several generations of Event MIR162 plants confirmed the expected Mendelian inheritance ratio for both *vip3Aa20* and *pmi* genes. »

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 (as given for applications EFSA/GMO/DE/2009/66 & 67)

No comments

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

Comments/Questions of the expert(s)

Comment 1 (as given for applications EFSA/GMO/DE/2009/66 and EFSA/GMO/DE/2009/67)

Is it 100% sure that a mutation occurred in the vip3Aa gene? It is not mentioned whether all sequenced clones contain the mutation (Appendix 4). Has sequencing been performed on PCR product (because it is known that Pfu polymerase also generates mutations during PCR)?
However the aa substition is conservative and therefore it can be anticipitated that this will have no effect on Vip3Aa protein function and toxicity.

Blastanalysis of the 5' and 3' flanking sequences:
This is not always clear. E.g. using the 5' flanking sequence, significant hits were obtained with two large clones. One should expect that the 3' flanking sequences would also show significant homology with these clones but apparently this is not the case. What is the reason for this?
Is the cyclophilin gene located on these two large clones?

New data compared to applications EFSA/GMO/DE/2009/66 & EFSA/GMO/DE/2009/66: Answer to question A.1 regarding MIR162 event updated bioinformatic search (from non CBI Appendix 36 and its appendix 1)

Comment 1

Reviewer agrees with the statement that event mir162 does not disrupt known genes in maize.

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

New data compared to applications EFSA/GMO/DE/2009/66 & EFSA/GMO/DE/2009/66: Answer to question A.2 regarding MIR162 event protein expression data (from non CBI Appendix 36 and its appendix 3)

Comments/Questions of the expert(s)

Comment 1

Reviewer agrees with the conclusions in the summary (p5) of appendix 3a of Appendix 36 and on page 11 of appendix 36:.

"The concentration levels of Vip3Aa20 and PMI were determined in the tropical hybrid corn MIR162 and its respective isogenic non genetically modified hybrid (non-GM), through the immuno-enzymatic assay ELISA, in different tissues from four development stages of plants cultivated in experiments conducted in Brazil, in the locations of Uberlândia-MG and Ituiutaba-MG, during 2007. The expression of the Vip3Aa20 protein was detected in measurable levels in all evaluated tissues, with the larger concentrations seen in the anthesis and whorl stages and decrease in the stages of seed maturity and senescence. For PMI the concentration was quantified in most of the tissues evaluated, with exception of leaf samples in the senescence stage, where the protein was not detected. The levels of expression of the proteins Vip3Aa20 and PMI in the respective transgenic hybrid isogenic non-GM (conventional) obtained measurements below the detection and quantification level in all samples of the evaluated tissues. »

"In summary, all different studies on expression levels of Vip3Aa20 and PMI proteins over 3 years (2005-2007), collected from four trials in the USA and two trials in Brazil and with different genetic backgrounds confirm that the ranges of the proteins are consistent in single event MIR162 maize.»

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

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

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

New data compared to applications EFSA/GMO/DE/2009/66 & EFSA/GMO/DE/2009/66: Answer to question B.1 regarding Compositional characteristics of MIR162 maize and its comparator(s) (from non CBI Appendix 36 and its appendices 4 and 5)

Comments/Questions of the expert(s)

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1 (as given for applications EFSA/GMO/DE/2009/66 and EFSA/GMO/DE/2009/67)

No further comments on the locations, growing seasons, geographical spreading and replicates.
The nutritional composition of whole grain kernels and maize forage derived from transgenic and isogenic maize was compared. This is in line with previous applications.

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1 (as given for applications EFSA/GMO/DE/2009/66 and EFSA/GMO/DE/2009/67)

The OECD guidelines were followed with respect to the selection of compounds. As it was the case in previous dossiers proximates, amino acids, fatty acids, minerals, vitamins, anti-nutrients and secondary metabolites were assessed in grain and proximates and selected minerals in forage.

D.7.4 Agronomic traits

New data compared to applications EFSA/GMO/DE/2009/66 & EFSA/GMO/DE/2009/66: Answer to question B.1 regarding Compositional characteristics of MIR162 maize and its comparator(s) (from non CBI Appendix 36 and its appendices 4 and 5)

Comments/Questions of the expert(s)

none

D.7.5 Product specification

Comments/Questions of the expert(s)

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1 (as given for applications EFSA/GMO/DE/2009/66 and EFSA/GMO/DE/2009/67)

The applicant refers to previous applications where processing according to dry and wet milling were studied. Key nutrients were analysed. As the transgenic maize is nutritionally equivalent to conventional maize no particular effects on processing are to be expected.
I agree with this conclusion.

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1 (as given for applications EFSA/GMO/DE/2009/66 and EFSA/GMO/DE/2009/67)

The proposed maize will replace some of the conventional maize. As no particular differences in composition have been demonstrated, no effects are to be expected.

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1 (as given for applications EFSA/GMO/DE/2009/66 and EFSA/GMO/DE/2009/67)

The number of animals was too small in order to be able a statistically significant difference in the reported trials with mice (2) and rats.

New data compared to applications EFSA/GMO/DE/2009/66 & EFSA/GMO/DE/2009/66: Answer to question B.5 regarding oral toxicity of VIP3Aa20 (from non CBI Appendix 36 and its appendix 11)

Comments/Questions of the expert(s)

Comment 1

Vip3Aa20: Sub-chronic Oral Toxicity (Appendix 11 of TECHNICAL DOSSIER For MIR162 maize)

Peng et al. (2007) identified a Vip protein produced from the novel Vip gene vip83 (GenBank Accession No. AY044227) from B. thuringiensis strain YBT-833 (Cai et al., 2002) and subsequently transformed and expressed the protein in a commercialized B. thuringiensis strain, which was identified as BMB696B. Peng et al. evaluated the toxicity of BMB696B and have published the results of both an acute and subacute (28-day) oral toxicity study in rats (Peng et al. (2007): this publication can be found in Appendix 11 of this response). The BMB696B protein shares 99.7% amino acid sequence homology with the Vip3Aa20 protein

Question: Did Peng et al. (2007) administer the purified VIP protein or was the powdered B. thuringiensis organism containing the protein used as test substance?

Page 1180 of this article states the following:

"…the recombinant plasmid was transformed and expressed in a commercialized B. thuringiensis strain YBT-1520, and then, a novel genetically modified B. thuringiensis strain BMB696B was obtained."

"Male and female Wistar rats were given BMB696B powder preparation which was made from this GM B. thuringiensis, at doses of 0, 500, 1250, 2500 or 5000 mg/kg bw by gavage for 14 in acute test and at doses of 0, 500, 1000 or 5000 mg/kg bw by gavage for 28 consecutive days in subacute test."

If no purified protein was used, then what are the administered dosis expressed as mg VIP protein/kg b.w. day?

D. 7.8.1 Safety assessment of newly expressed proteins

Comment 1 (as given for applications EFSA/GMO/DE/2009/66 and EFSA/GMO/DE/2009/67)

a) Degradation of the Vip3Aa20 protein in simulated gastric fluid (app 22; Stacy, 2007).

Vip3Aa20 from two sources, Event MIR162 transgenic maize and recombinant *Escherichia coli,* was readily degraded in SGF. No intact Vip3Aa20 (molecular weight *ca.* 89 kDa) from either source was detected following incubation in SGF for one minute, as assessed by Western blot analysis. An immunoreactive fragment of *ca.* 60 kDa was detected in the plant-expressed sample following

incubation in SGF for one minute. This protein fragment most likely represents a breakdown product of Vip3Aa20 due to pepsin action but was no longer detectable after 2 minutes of incubation in SGF for the plant-expressed protein.
The data presented in this report support the conclusion that Vip3Aa20 expressed in transgenic maize plants will be readily digested under typical mammalian gastric conditions.

c) Vip3Aa20: Acute Oral Toxicity Study in Mice (app 24; Draper, 2007).

Groups of five male and five female Alpk:APfCD-1 mice were dosed orally by gavage with 0 mg (control) or 1250 mg Vip3Aa20 protein/kg body weight (1488 mg MIR162VIP3A-0106 test substance/kg body weight) on a single day (as two fractions dosed 2 hours apart on day 1) using corn oil as the control substance and vehicle. Vip3Aa20 was the primary component of the test substance MIR162VIP3A-0106 (84% purity).

A dose of 1250 mg Vip3Aa20/kg body weight (equivalent to 1488 mg MIR162VIP3A-0106 test substance/kg body weight) administered orally was non-toxic to mice.

d) Vip3Aa20: Assessment of Amino Acid Sequence Homology with Known Toxins (app 20; Harper and Burroughs, 2009)

The BLASTP program was used to search the NCBI Entrez Protein Database to determine whether Vip3Aa20 had significant amino acid sequence similarity to known toxins. Of 57 protein sequences identified as having significant sequence similarity to Vip3Aa20, none were proteins known to be toxins other than insect-specific vegetative insecticidal proteins.

e) Degradation of the Phosphomannose Isomerase Protein protein in simulated gastric fluid (Privalle, 1999).

PMI was rapidly degraded in SGF such that no intact PMI was detected upon immediate sampling of the reaction mixture.
In order to demonstrate a time course of PMI degradation, the pepsin concentration in the SGF was reduced to 0.0001 times the standard concentration in a separate experiment. Under these conditions, both PMI protein and enzymatic activity were undetectable after 10 min at 37°C. These data indicate that PMI expressed in transgenic plants will likely be readily digested as conventional dietary protein under typical mammalian gastric conditions.

f) Degradation of the Phosphomannose Isomerase Protein protein in simulated intestinal fluid (Privalle, 1999).

PMI was rapidly degraded in SIF and no intact PMI was detected after 2 min of incubation at 37°C.

g) Phosphomannose Isomerase Protein: Acute Oral Toxicity Study in Mice (app 25; Kuhn, 1999).

Groups of seven male and six female mice were dosed orally by gavage with 3030 mg PMI protein/kg body weight in two doses, administered one hour apart. Groups of control males and females were also included. 0.5% w/v aqueous carboxymethylcellulose was used as the control substance and vehicle. Clinical observations and body weight were measured throughout the study. Fourteen days after dosing, the animals were sacrificed and subjected to an examination *post mortem*. Selected

organs were weighed. There were no treatment related effects of the PMI protein, therefore the acute oral LD_{50} as well as the no adverse effect level in mice was determined to be greater than 3030 mg PMI protein/kg body weight.

h) Phosphomannose Isomerase Protein: Assessment of Amino Acid Sequence Homology with Known Toxins (app 21; Harper, 2009)

The BLASTP program was used to search the NCBI Entrez Protein database to determine whether PMI had significant amino acid sequence similarity to known toxins. Of 580 sequences identified as having significant sequence similarity to PMI, none were proteins known to be toxins.

Comment 2 (as given for applications EFSA/GMO/DE/2009/66 and EFSA/GMO/DE/2009/67)

Proteins to be assessed: Vip3Aa20 protein and PMI protein (MIR162 maize).
No further comments.
The newly expressed proteins have been assessed well. There is no significant amino acid homology to known mammalian protein toxins and these proteins are readily degraded in *in vitro* digestibility assays. The Vip3Aa20 protein and PMI protein showed no acute toxicity in the single dose acute oral toxicity study in the mouse. A number of the tests were performed with Vip3Aa20 and PMI proteins produced by *E. coli*. The structural, biochemical and functional equivalence of the microbial substitute to the plant expressed proteins were clearly demonstrated.

New data compared to applications EFSA/GMO/DE/2009/66 & EFSA/GMO/DE/2009/66: Answer to question B.6 regarding simulated intestinal fluid assay with VIP3Aa20 (from non CBI Appendix 36 and its appendice 12)

Comments/Questions of the expert(s)

Comment 1

Degradation of the Vip3Aa20 protein in simulated intestinal fluid (Appendix 12 of TECHNICAL DOSSIER For MIR162 maize)

The SDS-PAGE analysis showed that Vip3Aa20 was rapidly digested in SIF. The degradation of Vip3Aa20 occurred so rapidly that no intact Vip3Aa20 was detected in the first sample taken after an incubation time of 5 min. However, a dominant protein band, corresponding to a molecular weight of ca. 62 kDa appeared immediately in the 5 min sample, slowly decreasing over the remaining time course but still detectable in the 48 hr sample.

Western blot analysis confirmed that the described 62 kDa band represents a degradation product of the Vip3Aa20 protein (due to the proteolytic activity of SIF) because it crossreacted with the anti-Vip3Aa antibodies. An additional immunoreactive band (molecular weight ca. 55 kDa) was also visible on the Western blot, after an incubation time of ca. 15 min in SIF, increasing in intensity over the time course and most likely represents a break down product derived from the described approximate 62 kDa band. The intensities of the protein bands as identified by SDS-PAGE and Western blot analyses (e.g. intensity of Vip3Aa20 in the zero-time sample in comparison to the 62 kDa band in the 5 min sample) showed that only a small portion of the Vip3Aa20 protein was converted into the described

break-down products and indicate that the major portion of the insecticidal protein will be completely degraded in SIF.

Conclusion: To my point of view, formation of these degradation products in SIF are of no concern due to the rapid degradation in SGF.

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Comment 1 (as given for applications EFSA/GMO/DE/2009/66 and EFSA/GMO/DE/2009/67)

No further comments.

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1 (as given for applications EFSA/GMO/DE/2009/66 and EFSA/GMO/DE/2009/67)

Only maize from one growing season (USA, 2005) was tested. Unfortunately no other controls than the corresponding non-transgenic, near-isogenic hybrid were used (no commercial control).

Forage
Statistically significant different (Stat. Sign. Diff.): Neutral Detergent Fiber (NDF) between genotypes. Average levels were within the ranges for conventional maize hybrids published by ILSI (2006)
Grain
Stat. Sign. Diff.: proximates ash, NDF, starch; minerals calcium, iron, phosphorus; vitamins A, B6, E; linoleic and linolenic fatty acids; secondary metabolites ferulic acid and ρ-coumeric acid. Average levels were within the ranges for conventional maize hybrids published by ILSI (2006) and OECD (2002).

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1 (as given for applications EFSA/GMO/DE/2009/66 and EFSA/GMO/DE/2009/67)

a) MIR162: 44-day feeding study in broiler chickens (**from CBI:** app 29; Brake, 2007).
Results of the broiler feeding study showed that neither the MIR162 grain, nor the non-transgenic, near-isogenic control grain fed broiler chickens demonstrated any adverse effects associated with consumption of poultry diets containing Vip3Aa20 or PMI compared to broiler chickens consuming diets made with commercially available grain containing no Vip3Aa20 or PMI. All diets supported rapid broiler chicken growth at low mortality rates and excellent feed conversion ratios without significant

impact on overall carcass yield or quality. The study showed that the transgenic maize had no deleterious effects on broiler chickens.

b) MIR162: 90-Day rat feeding study (**from CBI:** app 28; Barnes and Milburn, 2006).

Groups of twelve male and twelve female Alpk:APfSD (Wistar-derived) rats were fed diets incorporating Event MIR162 transgenic maize (corn) grain at 10.0% or 41.5% w/w, for at least 90 consecutive days.

There were no differences between groups of animals fed diets containing Event MIR162 positive transgenic maize grain or nontransgenic control maize grain in body weight, food consumption, clinical condition (including ophthalmoscopy and functional observation battery), clinical pathology, organ weights or histopathology that were considered to be attributable to the inclusion of the Event MIR162 positive transgenic maize grain in CT1 diet.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1 (as given for applications EFSA/GMO/DE/2009/66 and EFSA/GMO/DE/2009/67)

Assessment of the allergenicity of the newly expressed proteins.
According to the data currently available Vip3Aa is unlikely to be allergenic.
About MIR604 PMI, in a previous dossier (EFSA/GMO/UK/2007/50, maize Bt11xMIR604), the applicant described possible cross-reactivity with a moderately important latex allergen, Hev b 13, the homology being between 29.6% and 36.2%, depending on the comparative method. This was not considered a significant allergen homology as per the guidelines set by the Codex Alimentarius Commission (2003)."
Although such PMI-Hev b 13 homology is not found in the present dossier, the reviewer still finds that 29.6% of homology represents enough amino-acids to construct several cross-reactive epitopes with Hev b 13. Therefore, it is relevant to evaluate the reactivity of PMI on patients allergic to Hev b 13 by using in vivo (skin tests) and/or in vitro (IgE binding) techniques.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1 (as given for applications EFSA/GMO/DE/2009/66 and EFSA/GMO/DE/2009/67)

The number of pens was too small in the broiler trial in order to be able to find a statistically significant difference with respect to feed conversion ratio. The large variability with respect to dry air temperature during the trial might have interfered with the performance results.

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

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

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)

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

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)

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

References

None