Bioveiligheidsraad
Conseil de Biosécurité

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Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2010_0958

Title: Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/UK/2008/56 from Syngenta under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/UK/2008/56 was submitted by Syngenta on 21 May 2008 for the marketing (import and processing) of the insect resistant, glyphosate and glufosinate-tolerant genetically modified Bt11 x MIR604 x GA21 maize for food and feed uses under Regulation (EC) No. 1829/2003[1].

The application was officially acknowledged by EFSA on 19 August 2008. On the same date EFSA started the formal three-month consultation period of the Member States, in accordance with Articles 6.4 and 18.4 of Regulation (EC) No. 1829/2003 (consultation of national Competent Authorities within the meaning of Directive 2001/18/EC designated by each Member State in the case of genetically modified organisms (GMOs) being part of the products).

Within the framework of this consultation, the Belgian Biosafety Advisory Council, under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier, chosen from the common list of experts drawn up by the Biosafety Advisory Council and the Division of Biosafety and Biotechnology (SBB). Eight experts answered positively to this request, and formulated a number of comments to the dossier, which were edited by the coordinator. See Annex I for an overview of all the comments and for the list of comments actually placed on the EFSAnet on 13 November 2008.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 29 April 2010 (The EFSA Journal, 2010, 8 (5):1616)[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 20 May 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 end October 2008 was provided to the coordinator and to the experts who evaluated the toxicological and allergenic aspects of this GM maize. See Annex II for an overview of all the comments transmitted by the experts.

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. (OJ L 268, 18.10.2003, p.1)
[2] See: http://www.efsa.europa.eu/en/scdocs/scdoc/1616.htm

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The comments formulated by the experts together with the opinion of EFSA including the answers of the EFSA GMO Panel form the basis of the advice of the Biosafety Advisory Council given below.
In addition, the latest scientific evaluations of the single events, namely maize line MIR604 (EFSA/GMO/UK/2005/11), maize line Bt11 (EFSA/GMO/RX-Bt11) and maize line GA21 (EFSA/GMO/UK/2005/19), are taken into account in this advice. Due to concerns about the potential allergenicity of the MIR604 maize the Biosafety Advisory Council formulated a negative advice for MIR604 GM maize. For Bt11 maize the Biosafety Advisory Council formulated a positive advice and for GA21 maize due to shortcomings in the scientific quality of the data the Biosafety Advisory Council could not conclude on the safety of this GM event[3].

Scientific evaluation

1. Environmental risk assessment

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

2. Molecular characterisation

With regard to the molecular characterisation, 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.

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

A majority of the members of the BAC supports the following opinion :

New data presented by the company do not take away all reserves regarding potential allergenicity of the transgene protein. Although the 29,6% homology between the MIR604 PMI and Hev b13 (a known allergen) is below the 35% level, used by the Codex Alimentarius, the company failed to convince the members of the BAC by presenting exclusively data on sequence homology and by not presenting appropriate data from *in vitro* and/or *in vivo* tests which could have taken away the doubts.
In addition, the Biosafety Advisory Council recommends following up any unanticipated allergenicity aspects of the GM plant in monitoring systems.

[3] Advice of BAC on maize line MIR604: BAC_2009_01365; Advice of BAC on maize line Bt11: BAC_2009_904; Advice of BAC on maize line GA21: BAC_2007_SC_614
[4] As the application doesn't imply a cultivation of the GM crop in the EU, a full environmental assessment is not required in EFSA procedure and was not achieved.

A minority of the members supports the following opinion :

The fact that the PMI protein shows a 29,6% homology with the Hev b 13 latex protein has correctly resulted in questions to provide additional information on its potential allergenicity. The additional data provided by the notifier mostly concern in silico analyses, but also include the results of an IgE binding test to frog alpha-parvalbumin. Taken together with the fact that the PMI protein is fully identical to native E.coli PMI protein, and where E.coli is not known to cause allergic reactions and is abundantly present in the human gut, this results in a extremely low probability of the PMI protein to cause any allergic reactions.

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
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 Bt11 x MIR604 x GA21 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 tested *in vitro* on serum of patients allergic to latex nor by appropriate *in vivo* tests.

The BAC therefore cannot give a univocal conclusive advice for the placing on the market of the insect-resistant genetically modified maize Bt11 x MIR604 x GA21.

M. Goossens

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

Annex I: Full comments of experts in charge of evaluating application EFSA/GMO/UK/2008/56 *and Comments submitted on the EFSAnet (ref. BAC_2008_840)*
Annex II: Compilation of comments of experts in charge of evaluating the additional information received for application EFSA/GMO/UK/2008/56 (ref.BAC_2010_831)

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13-11-2008

Compilation of comments of experts in charge of evaluating the application EFSA/GMO/UK/2008/56
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 2008
Coordinator: Prof. dr. ir. Dirk Reheul
Experts: Pascal Cadot (Consultant), Leo Fiems (ILVO), Rony Geers (KUL), Lieve Gheysen (UGent), Jean-Luc Hofs (FUSAGx), Peter Smet (Consultant), Wim Stevens (UIA), Hadewijch Vanhooren (KUL)
Domains of expertise of experts involved: Genome analysis, genetic engineering, animal nutrition, traceability of alimentary chain, toxicology, immunology, alimentary allergology, agronomy, ecology, bio-diversity, plant-insect relations, insect resistance, herbicide tolerance, biosafety research, maize.
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

INTRODUCTION

Dossier **EFSA/GMO/UK/2008/56** concerns an application of the company **Syngenta seeds** for the marketing of the genetically modified **maize Bt11xMIR604xGA21** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 19 August 2008.
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. Comments placed on the EFSAnet are indicated in grey.

List of comments received from the experts

GENERAL COMMENTS

Comment 1

Problems are not expected from the import of feed and food products derived from Bt11 x MIR604 x GA21 maize, because:

- Bt11 maize has already received approval for import food and feed use in the EU
- GA21 maize is approved for food use
- the import and use of Event MIR604, expressing mCry3A poses no foreseeable human health risks (EPA, 2007)
- the concentrations of transgenic proteins in Bt11 × MIR604 × GA21 maize plant tissues seems similar to the concentrations of these proteins in tissues of the corresponding single-event hybrid maize plants

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1

Agreement with the provided information.

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

Comments/Questions of the expert(s)

Comment 1

Section B.2., (a) reproduction: information about pollen viability reflects minimum or regular pollen field life but do not consider maximum measurements. Brunet (2006) showed that maize pollen grains are transported by air convection at a non negligible altitude where thermo-hydric conditions allow an increase of their viability by hours. Luna *et al*. (2001) calculated a theoretical maximum pollen viability distance of 32 km.

Section B.3 .: Even though cultivation is not in the scope of this application and survivability of maize in Europe is very limited, it is important to mention that survivability should be monitored in Southern Europe where winter average temperatures are close to 15°C (South West Spain and SW Sicily).

Additional comment from the coordinator

Section B.2.,
The dossier should be completed with an update of research on pollen flow. There are some excellent reviews available.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Comment 1

Information is satisfactory.

Comment 2

None

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

Information is complete.

Comment 2

None

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

Comments/Questions of the expert(s)

Comment 1

Appendix 1 demonstrates that transgenic insert integrity in the stacked plant has been preserved.

Comment 2

According to the guidelines for stacked events, Southern blot analyses have been performed to confirm the presence, copy number and configuration of each of the events derived from the three parent transgenic lines in the stacked line. The southern blot analyses are complicated because of the combination of three events, but they are well described with for each event 1. a scheme of the inserted DNA, 2. a predicted restriction map of the transgene insert and the location of the probes used, 3. a table with a summary of the expected and the observed fragments and 4. the actual southern results. These results clearly demonstrate that the transgene inserts have correctly been inherited from the parent lines.

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1

Assessment has been performed according to EFSA-Q-2003-005-D response related to the guidance document on GM plant containing stacked transformation events. Information is satisfactory.

Comment 2

Although some statistically significant differences were seen, these differences were small or not consistent across the growing season. In any case, if differences occurred, the expression was slightly lower in the stacked line, excluding possible effects on changes in toxicity. These results support the conclusion that transgenic protein expression in Bt11 x MIR604 x GA21 maize is not substantially different from that of the Bt11, MIR604 or GA21 single maize events.

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

The report confirms that no changes in reproduction, dissemination and survivability happen compared with the check.

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

Comments/Questions of the expert(s)

Comment 1

Segregation data show Mendelian genetic patterns for all inserted genes. We can thus conclude for the stability and normal heritability of the inserted genes.

Comment 2

Since the plants will not be grown in the EU, stability tests over generations are not required.

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

Comments/Questions of the expert(s)

Comment 1

Transfer of genetic materials to other organisms is nil or little likely with very low impact for some of them.

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

a) maize forage

Proximates.
There were no statistically significant differences in moisture, protein, ash, carbohydrates, ADF and NDF. A significant location-by-genotype interaction was observed for fat. The mean levels of all proximates across locations and for each location were within the ranges reported in the ILSI database.

Phosphorus and calcium.
Phosphorus levels did not differ significantly between the two genotypes. A significant location-by-genotype interaction was observed for calcium. The mean levels of both minerals across locations and for each location were within the ranges reported in the ILSI database.

b) maize grain

Proximates.
There were no statistically significant differences in fat, carbohydrates, ADF, NDF, TDF and starch. A statistically significant difference in protein levels between the genotypes was observed. Also, a significant location-by-genotype interaction for ash. The mean levels of all proximates across locations and for each location were within the ranges reported in the ILSI database.

Minerals.
Levels of copper, iron, magnesium, manganese, phosphorus, potassium, and selenium did not differ significantly between the two genotypes. Zinc levels were statistically significant different and a significant location-by-genotype interaction was observed for calcium. For sodium, levels below the limit of quantitation (LOQ) precluded statistical analysis. Mean levels of all minerals across locations and for each location were within the ranges reported in the ILSI database.

Vitamins.
Levels of vitamins A, B2, B3, B6, and B9 did not differ significantly between the genotypes. A statistically significant difference was observed for vitamin B1. For vitamin E, levels below the limit of quantitation (LOQ) precluded statistical analysis. All mean levels across locations and for each location were within the ranges reported in the ILSI database except for vitamin B2 levels, which were slightly higher in the transgenic grain at location 8 and in the nontransgenic grain at location 1, and the vitamin E levels that were <LOQ in both transgenic and nontransgenic grain at some locations. Below LOQ values for vitamin E are not represented in the ILSI database.

Amino Acids.
Most of the amino acid levels differed significantly between the genotypes, a result consistent with the difference in protein levels. All mean amino acid levels across locations and for each location were within the ranges reported in the ILSI database.

Fatty Acids.
The proportion of the five most abundant fatty acids, as a fraction of total fatty acids did not differ significantly between the genotypes. All mean levels across locations and for each location were within the ranges reported in the ILSI database.

Secondary Metabolites and Antinutrients.
Levels of ferulic acid, *p*-coumaric acid, inositol, phytic acid, and trypsin inhibitor did not differ significantly between the genotypes. Levels of raffinose and furfural below the LOQ precluded statistical analysis. All mean levels across locations and for each location were within the ranges reported in the ILSI database

Comment 2

No comment

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

Bt11xMIR604xGA21 was treated with Glyphosate and Glufosinate-ammonium. There are no indications about the herbicide active ingredient concentration, dose, total load, number of sprays on the plants. This agronomic information should be detailed, but is not given in in Appendix 4. The information should be added.

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

No comment

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

Information on crop maintenance in Appendix 3 doesn't report numbers and dates of herbicide sprays performed on Bt11xMIR604xGA21. This information should be added.

Pollen viability trait related to herbicide use on the crop should have been discussed as this point seems to be controversial. Some sources state that some detrimental interactions can occur (Pline-Srnic, 2005) others conclude to the absence of effects (Thomas et al., 2004).
Question: Does Glyphosate or Glufosinate-ammonium repeated treatments on Bt11xMIR604xGA21 event affect pollen viability?

Comment from SBB

As CULTIVATION is not within the scope of this application, the effect of Glyphosate or Glufosinate-ammonium on pollen viability is not really pertinent for this dossier.

D.7.5 Product specification

Comments/Questions of the expert(s)

Comment 1

No comment

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1

No comment

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

No comment

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

This application involves a stacking of two approved events (BT11, GA21) and one event pending for approvement (MIR604). As such, the risk assessment of the two approved events (BT11, GA21) can be used in the risk assessment of the stacked BT11 x MIR604 x GA21 maize. However, as the risk assessment of the MIR604 event is not completed yet, the application of the stacked event BT11 x MIR604 x GA21 maize should be supplemented with the comprehensive evaluation of the MIR604 event.
More specific, for toxicological safety assessment the reviewers should be provided with the toxicity studies of the MIR604 event, including studies on the newly expressed proteins (molecular and biochemical characterisation, stability testing, resistance to proteolytic enzymes, sub-acute 28-day oral toxicity study), and including the testing of the whole GM food/feed 90-days rodent feeding study. A search for homology to toxins was submitted.

Comment from SBB

We can refer here to the comment that the expert had written in February 2006 when evaluating dossier EFSA/GMO/UK/2005/11 (Maize MIR604):

Screening for structure-activity relationship, *in vitro* digestibility assays, and acute toxicity testing.
A battery of tests showed that the newly expressed proteins, mCry3A and PMI, are structurally and functionally not related to known toxins (App. XI and XVII) and food allergens (App. XII and XVIII). The mCry3A and PMI proteins were found sensitive to processing and heat (App. V and XIV; App. XX) and are rapidly degraded under simulated mammalian gastric and intestinal conditions (App. XIII and XIX). In the acute oral toxicity studies of the mCry3A protein (2377 mg/kg bw) and PMI protein (5050 mg/kg bw) in the mouse, no signs of systemic toxicity were found. There were no test-substance related effects on bodyweight, food consumption, organ weight or macroscopic or microscopic pathology (App. VI and VIII). The proteins were found not acutely toxic to mice.
The proteins used for the *in vitro* digestibility studies and the acute oral toxicity studies were produced by recombinant E. coli. The test substances PMI-0198 (App. II) and MCRY3A-0102 (App. VII, XV, XVI) were compared by various structural, functional and biochemical parameters to the PMI protein and the mCry3A protein produced in the transgenic maize event MIR604. The proteins from recombinant E. coli and the MIR604-derived maize are found substantially equivalent and the microbial test substances can be accepted as a suitable surrogate.

Repeated dose toxicity testing.
No 28-day oral toxicity test has been performed with either proteins. It was motivated by the applicant that since no safety concerns have been raised by the acute toxicity studies, digestibility studies or toxin homology searches, both proteins can be considered non-toxic and unlikely to present a health risk to humans and animals.
We cannot completely agree with this motivation, but as whole-food toxicity testing has been performed consisting of a well-conducted and well-documented 90-days feeding study in rats (including biochemical, haematological and histological endpoints) and a 49-days broiler chicken study, we decide not to request for a 28-days oral toxicity study combining the 2 proteins.
Genotoxicity testing.
Genotoxicity testing, whether it is performed or not, should always be well motivated.

Comment 2

All three single GM maize events have been previously compared to their respective near-isogenic conventional maize lines for their toxic potential on humans and animal health. The results of these evaluations provide evidence to conclude that Bt11, MIR604 and GA21 maize are not different to conventional maize and are therefore unlikely to be toxic.
A feeding experiment with broilers, lasting 49 days, including from ±50% maize in the starting period to more than 60% in the finishing period, did not show detrimental effects.

Comment 3

The use of Herbicide "over-the –top" application linked with the genetic modification should be assessed for glyphosate residues and AMPA metabolite, as well as the surfactant residues. The applicant should demonstrate (or discuss) the innocuousness of the specific herbicide tolerant crop management on human and animal health. Biotechnological assessment should be combined with a specific pesticide risk assessment related to the molecules contained in the herbicides used on the transgenic plant.

Comment from SBB

*The effect of herbicides residues on human or animal health falls under Directive **91/414/EC**.*

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

See preceding comment concerning the MIR604 event: The reviewers should be provided with the toxicity studies of the MIR604 event, including studies on the newly expressed proteins (molecular and biochemical characterisation, stability testing, resistance to proteolytic enzymes, sub-acute 28-day oral toxicity study), and including the testing of the whole GM food/feed 90-days rodent feeding study.
A search for homology to toxins was submitted for the five transgenic proteins.

Comment 2

Protein concentration units are µg/g DW. N = 5 for pollen (one pooled sample per replicate block) and 10 for all other tissues (two samples per replicate plot), unless otherwise noted. Results significantly different at F-test Probability <5% are shown in bold, italic type.

Protein	Hybrid	Statistic	Leaves	Roots	Pollen	Kernels	Whole Plants
Cry1Ab	Bt11	mean	32.5	10.3	–	1.4	18.8
		range	26.8–40.6	8.0–12.7	<0.5[b]	0.8–1.8	13.0–21.3
	Stacked[a]	mean	29.4	8.8	–	1.6	19.0
		range	26.1–35.4	6.9–10.4	<0.5[b]	0.6–1.9	15.3–22.5
		SD	1.7	0.5	–	0.3	1.5
		F-Test Probability	8.2%	***3.1%***	–	39.0%	85.7%
PAT	Bt11	mean	0.6	1.0	–	–	0.7
		range	0.5–0.7	0.6–1.3	<0.4[c]	<1.6[d]	0.7–0.8
	Stacked[a]	mean	0.6	0.8	–	–	0.9
		range	0.6–0.8	0.7–1.0	<0.4[c]	<1.6[d]	0.6–1.0
		SD	0.0	0.1	–	–	0.1
		F-Test Probability	5.2%	7.0%	–	–	7.7%

– = not applicable. Leaf, root, pollen, and whole-plant samples analyzed were collected at the anthesis stage. The kernel samples analyzed were collected at the physiological maturity stage. [a]N = 8 for all stacked hybrid leaf and root samples; N = 9 for all stacked hybrid whole-plant samples. [b]0.5 µg/g DW = LOQ. [c]0.4 µg/g DW = LOD. [d]1.6 µg/g DW = LOQ.

These data are comparable to those provided in dossier RX-Bt11 ((Bt11 maize).

The amounts of both proteins in the stacked event are indeed comparable to those of the comparator (Bt11)

Protein concentration units are µg/g DW. N = 5 for pollen (one pooled sample per replicate block) and 10 for all other tissues (two samples per replicate plot), unless otherwise noted. Results significantly different at F-test Probability <5% are shown in bold, italic type.

Protein	Hybrid	Statistic	Leaves	Roots	Pollen	Kernels	Whole Plants
mCry3A	MIR604[a]	mean	34.1	17.4	–	–	15.3
		range	28.8–39.7	11.3–25.5	<0.8[c]	<0.6[d]	11.3–20.0
	Stacked[b]	mean	38.1	18.8	–	–	18.0
		range	29.8–51.0	12.9–21.8	<0.8[c]	<0.6[d]	13.6–20.5
		SD	5.1	2.1	–	–	2.7
		F-Test Probability	34.0%	41.2%	–	–	19.8%
MIR604 PMI	MIR604[a]	mean	14.7	5.2	74.3	2.9	10.0
		range	11.7–16.3	3.8–6.5	68.3–90.8	1.4–4.8	8.0–12.5
	Stacked[b]	mean	14.2	4.6	73.3	3.1	11.2
		range	12.6–14.7	3.5–5.8	65.5–86.8	2.1–4.9	10.1–12.1
		SD	1.1	0.7	6.9	1.0	1.0
		F-Test Probability	52.4%	30.3%	83.7%	77.1%	13.0%

– = not applicable. Leaf, root, pollen, and whole-plant samples analyzed were collected at the anthesis stage. The kernel samples analyzed were collected at the physiological maturity stage. [a]N= 9 for all MIR604 hybrid leaf, root, and kernel samples. [b]N= 8 for all stacked hybrid leaf and root samples; N= 9 for all stacked hybrid whole-plant samples. [c]0.8 µg/g DW = LOQ. [d]0.6 µg/g DW = LOQ.

These data are comparable to those provided in dossier 48 (MIR604 x GA21 maize).

The amounts of both proteins in the stacked event are indeed comparable to those of the comparator (MIR604)

Protein concentration units are µg/g DW. N = 5 for pollen (one pooled sample per replicate block) and 10 for all other tissues (two samples per replicate plot), unless otherwise noted. Results significantly different at F-test Probability <5% are shown in bold, italic type.

Protein	Hybrid	Statistic	Leaves	Roots	Pollen	Kernels	Whole Plants
mEPSPS	GA21	mean	32.4	17.7	178.8	6.8	21.5
		range	26.4–37.8	13.6–21.2	96.7–194.7	5.2–8.5	19.4–23.2
	Stacked[a]	mean	27.1	10.5	171.8	6.3	21.5
		range	22.6–31.3	8.6–15.2	163.4–179.6	5.3–7.1	16.8–23.9
		SD	2.5	2.0	7.4	0.6	0.8
		F-Test Probability	5.7%	***1.5%***	27.5%	25.2%	93.6%

Leaf, root, pollen, and whole-plant samples analyzed were collected at the anthesis stage. The kernel samples analyzed were collected at the physiological maturity stage. [a]N = 8 for all stacked hybrid leaf and root sample; N= 9 for all stacked hybrid whole-plant samples.

These data are comparable to those provided in dossier 49 (Bt11 x GA21 maize).

The amounts of both proteins in the stacked event are indeed comparable to those of the comparator (GA21)

a) Degradation of the Cry1Ab protein in simulated gastric fluid. (from RX-Bt11)

Rapid digestion was demonstrated previously. **No further testing is needed.**

b) Degradation of the Cry1Ab protein in simulated intestinal fluid. (from RX-Bt11)

Not mentioned. Has this test been performed? If not, why not?

c) Degradation of the PAT protein in simulated gastric fluid. (from RX-Bt11)

Rapid digestion was demonstrated previously. No further testing is needed.

d) Degradation of the PAT protein in simulated intestinal fluid. (from RX-Bt11)

Not mentioned. Has this test been performed? If not, why not?

e) Degradation of the Cry3A protein in simulated gastric fluid (author). (from MIR604 x GA21)

I did not evaluate dossier 11. The technical dossier states that the mCry3A protein is readily degraded in in vitro digestibility assays.

f) Degradation of the Cry3A protein in simulated intestinal fluid (author). (from MIR604 x GA21)

I did not evaluate dossier 11. The technical dossier states that the mCry3A protein is readily degraded in in vitro digestibility assays.

g) Degradation of the PMI protein in simulated gastric fluid (author). (from MIR604 x GA21)

Not mentioned. Has this test been performed? If not, why isn't it performed?

h) Degradation of the PMI protein in simulated intestinal fluid (author). (from MIR604 x GA21)

Not mentioned. Has this test been performed? If not, why isn't it performed?

i) Degradation of the mEPSPS protein in simulated gastric fluid (author). (from MIR604 x GA21)

Test was previously performed. Rapid digestion was demonstrated.

j) Degradation of the mEPSPS protein in simulated intestinal fluid (author). (from MIR604 x GA21)

Test was previously performed. Rapid digestion was demonstrated.

k) Cry1Ab: Acute Oral Toxicity Study in Mice (Finlay, 2006). (from RX-Bt11)

No toxic effects have been observed in acute toxicity studies done with test material derived from microbial cultures biochemically and insecticidally similar to the delta-endotoxin as produced by the Bt11 maize. **No further testing is needed.**

l) PAT: Acute Oral Toxicity Study. (from RX-Bt11)

Lack of acute toxicity was demonstrated earlier. **No further testing is needed.**

m) Cry3A: Acute Oral Toxicity Study in Mice (author). (from MIR604 x GA21)

Lack of acute toxicity was demonstrated earlier. **No further testing is needed.**

n) PMI: Acute Oral Toxicity Study in Mice (author). (from MIR604 x GA21)

Lack of acute toxicity was demonstrated earlier. **No further testing is needed.**

o) mEPSPS: Acute Oral Toxicity Study in Mice (author). (from MIR604 x GA21)

Lack of acute toxicity was demonstrated earlier. **No further testing is needed.**

p) Cry1Ab: Assessment of Amino Acid Sequence Homology with Known Toxins

The NCBI Entrez protein database was searched using the BLASTP program to determine if the Cry1Ab protein has any significant amino acid sequence homology to known toxins. It was concluded

that, except for the expected sequence homology to other delta-endotoxins including other Cry proteins, the Cry1Ab query sequence showed no significant sequence homology to any proteins identified as, or known to be, toxins.

q) PAT: Assessment of Amino Acid Sequence Homology with Known Toxins

The NCBI Entrez protein database was searched using the BLASTP program to determine if the phosphinothricin acetyltransferase protein (PAT) has any significant amino acid sequence homology to known toxins. It was concluded that the PAT query sequence showed no significant sequence homology to any proteins identified as, or known to be, toxins.

r) Cry3A: Assessment of Amino Acid Sequence Homology with Known Toxins

The BLASTP program was used to search the NCBI Entrez Protein database to determine whether mCry3A had significant amino acid sequence similarity to known toxins. The threshold value for statistical significance of matches was based on searches conducted with randomly shuffled sequences of the amino acids comprising mCry3A. Of 308 sequences identified as having significant sequence similarity to mCry3A, none were proteins known to be toxins other than delta-endotoxins, including other Cry proteins.

s) PMI: Assessment of Amino Acid Sequence Homology with Known Toxins

The NCBI Entrez protein database was searched using the BLASTP program to determine if the phosphomannose isomerase protein as expressed in transgenic Event MIR604 maize (corn) (MIR604 PMI) has any significant amino acid sequence homology to known toxins. It was concluded that the MIR604 PMI query sequence showed no significant sequence homology to any proteins identified as, or known to be, toxins.

t) mEPSPS: Assessment of Amino Acid Sequence Homology with Known Toxins

The NCBI Entrez protein database was searched using the BLASTP program to determine if the double mutated maize 5-enol pyruvylshikimate-3-phosphate synthase protein (mEPSPS) has any significant amino acid sequence homology to known toxins. It was concluded that the mEPSPS query sequence showed no significant sequence homology to any proteins identified as, or known to be, toxins.

Comment 2

The expressed proteins have a history of safe use. Information provided is satisfactory.

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Comment 1

No further comments.

Comment 2

Information provided is satisfactory.

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1

No further comments.

Comment 2

No comment

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

Although a broiler feeding study was conducted (broiler feeding studies are focused on adverse effects due to nutritional changes), additional risks which may rise from the combined effects of the stacked genes should still be evaluated by a whole food toxicity study: the 90-days feeding study in rodents with complete endpoints adapted from the OECD 90-days rodent toxicity study (OECD guideline 408) with special attention to the selection of doses and the avoidance of problems of nutritional imbalance.
Minor comment on the 49-days broiler feeding study: The transgenic maize grain (lot WN-R63440) and non-transgenic, near isogenic maize (lot WN-R64180) grown in the 2006 season, were not from the same field trials as the maize grown in the compositional study and the agricultural study.

Additional comment from the coordinator

Applicants should use completely comparable material to conduct their feeding trials !

Comment 2

a) 49-day feeding study in broiler chickens (Brake, 2008).
Final overall mean body weights of the Bt11 x MIR604 x GA21 group, the nontransgenic group, and the NC 2007 group did not differ significantly. There were no significant differences in cumulative feed conversion ratios to 49 days of age among broilers fed diets containing the three different sources of maize grain. During the grower period (days 16 to 35), broilers fed Bt11 x MIR604 x GA21 diets had improved feed conversion compared with broilers fed NC 2007 diets, but were not significantly different than broilers fed the nontransgenic diets. There was an interaction between maize source

and sex during the finisher period (days 35 to 49) due to inconsistent differences between males and females within a type of maize at various times (There were sporadic maize grain source-by-sex interactions for both unadjusted and adjusted feed conversion efficiency at various times due to inconsistent differences between sexes within maize grain types). Overall bird survival was good (>97%), and there were no effects on mortality due to either maize grain or sex of the birds. There were no significant differences in carcass yield due to maize grain source among males and females.

b) 90-Day rat feeding study (author).

Not performed. No further testing is needed.

Comment 3

No comment

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

Bt11 x MIR604 x GA21 maize was produced by combining Bt11, MIR604 and GA21 maize through conventional breeding.
- Cry1Ab which confers protection against certain lepidopteran pest species
- PAT which confers tolerance to herbicide products containing glufosinate ammonium.
- mCry3A which confers protection against certain coleopteran pest species
- MIR604 PMI which acts as a selectable marker, its expression allows maize plant cells to grow under artificial conditions
- mEPSPS confers tolerance to herbicide products containing glyphosate

Maize as such is not very allergenic.
The allergenicity of the three proteins has already been evaluated in the dossier EFSA/GMO/UK/2007/49.
- An extensive bioinformatics search for sequence homologies and structural similarities between the expressed proteins and known allergens was performed. The results demonstrated that Cry1Ab, PAT and mEPSPS proteins show no homology to any known or putative allergenic proteins.
- The susceptibility of Cry1Ab, PAT and mEPSPS proteins to proteolytic degradation was evaluated in simulated mammalian gastric fluid (SGF) containing pepsin. All the proteins were readily degraded in SGF. No intact or immunoreactive fragments were detected following digestion in SGF for 2 minutes. These data support the conclusion that Cry1Ab, PAT and mEPSPS expressed in transgenic plants will be readily digested as conventional dietary protein under typical mammalian gastric conditions.

According to Mayet (2005), quoting Gendel (1998), the Cry3A similarity to a known food allergen found in cow's milk, beta-lactoglobulin, suggests a possibility that mCry3A is a potential allergen.

Some general remarks have to be made:
allergenicity is an individual trait that cannot be predicted; any novel protein or polypeptide has potential allergenic properties which will only be discovered after meticulous follow up the digestibility of proteins is only a relative capacity as far as allergenicity is concerned since proteins can as such induce allergic reactions before biotransformation as has been demonstrated for apple, carrot, potato and other allergens.

Concerning the MIR604 PMI protein: in an assessment of the UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (2006) the similarity between the PMI protein and parvalbumin is negligible. However caution is mandatory since parvalbumin is increasingly recognized as an allergen (Hilger 2004). In the report on dossier EFSA/GMO/UK/2007/50 it was already stated that "*A sequence identity greater than 35% between one of the sequential MIR604 PMI 80-amino acid peptides and an allergen from Hevea brasiliensis (Hev b 13) was also found. However close comparison of MIR604 PMI and Hev b 13 led to the conclusion that the elements that were responsible for the allergenicity of Hev b 13 were not present in MIR604 PMI and it could therefore be concluded that MIR604 PMI was unlikely to be an allergen".*
Here too caution is mandatory and follow-up is necessary.

Comment 2

Assessment of the allergenicity of the newly expressed proteins.
According to the data currently available, Cry1Ab, mCry3A, PAT and mEPSPS are unlikely to be allergenic.
About MIR604 PMI, the applicant refers to a dossier where it was shown to possibly cross-react with alpha-parvalbumin from *Rana* species. This, however, was correctly shown by the applicant, with patient serum testing, to be non-relevant.
In another dossier (EFSA/GMO/UK/2007/50, maize Bt11xMIR604), the applicant described possible cross-reactivity with a moderately important latex allergen, Hev b 13, with MIR604 PMI – Hev b13 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).
In the present dossier, PMI-Hev b 13 homology is not found (appendix 12); what is the reason for such discrepancy?
Nevertheless, on the basis of the previous applications with the same MIR604 strain, the reviewer still finds that 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.

Assessment of the allergenicity of the whole GM plant or crop.
The applicant did not assess the allergenicity of the whole GM plant. Conversely to what is stated in the application, maize allergy is documented, although it is not recognized as a major allergy concern. Some maize allergens have been described in the literature (Pastorello et al. 2003; Pasini et al. 2002, Weichel et al. 2006). Recently, patients showed maize-induced anaphylaxis, and some reacted 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.

Due to the introduction of the five new traits as described in the application, over-expression of endogenous proteins, among them possibly the maize allergens, may occur. Therefore, 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, compared to a natural counterpart. Patient IgE binding to maize grain extract or titration of known major allergens of maize should be carried out.

Comment 3

All three single GM maize events have been previously compared to their respective near-isogenic conventional maize lines for their allergenic potential. These evaluations provide evidence to conclude that Bt11, MIR604 and GA21 maize are not different to conventional maize and are therefore unlikely to be allergenic.

Summary of the above comments as made by the coordinator:

1) According to Mayet (2005), quoting Gendel (1998), the Cry3A has similarity to a known food allergen found in cow's milk, beta-lactoglobulin, suggests a possibility that mCry3A is a potential allergen.
2) Concerning the MIR604 PMI protein: in an assessment of the UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (2006) the similarity between the PMI protein and parvalbumin is negligible. However caution is mandatory since parvalbumin is increasingly recognized as an allergen (Hilger 2004).
3) In the report on dossier EFSA/GMO/UK/2007/50 it was stated that "*A sequence identity greater than 35% between one of the sequential MIR604 PMI 80-amino acid peptides and an allergen from Hevea brasiliensis (Hev b 13) was found. But in the present dossier PMI-Hev b 13 homology is not found. What is the reason for this discrepancy ?*

Our experts have a slightly different opinion on the relevance and the importance of these possible allergens. In particular, the homology noticed in 3) should be considered with most care.
Therefore it is :
1) highly recommended to test the reactivity of these proteins on patients with known relevant allergies by using in vivo (skin) tests or in vitro (IgE binding) techniques.
2) to organise a follow up after the introduction of these proteins and to warn people on the presence of them.

Moreover, ***the applicant did not assess the allergenicity of the whole GM plant.*** Conversely to what is stated in the application, maize allergy is documented, although it is not recognized as a major allergy concern. Some maize allergens have been described in the literature (Pastorello et al. 2003; Pasini et al. 2002, Weichel et al. 2006). Recently, patients showed maize-induced anaphylaxis, and some reacted 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.
Due to the introduction of the five new traits as described in the application, over-expression of endogenous proteins, among them possibly the maize allergens, may occur. Therefore, 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, compared to a natural counterpart. Patient IgE binding to 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

No unexpected alterations in nutrients and other feed components have occurred.
All three single GM maize events have been previously compared to their respective near-isogenic conventional maize lines for their nutritional effects on humans and animal health. The results provide evidence that Bt11, MIR604 and GA21 maize are not different to conventional maize and their nutritional effects are no different to those for conventional maize.
A feeding experiment with broilers, lasting 49 days, including from ±50% maize in the starting period to more than 60% in the finishing period, showed no negative effects on growth rate and overall carcass yield and quality.

Comment 2

No comment

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

Comments/Questions of the expert(s)

Comment 1

No comment

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

Comments/Questions of the expert(s)

Comment 1

No comment

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

There is no strong evidence that Bt11xMIR604xGA21 event will develop persistence and invasiveness.

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

Comment 1

No comment

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

Comment 1

No comment

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

Comment 1

As feral populations are improbable in most of the European environments, there is no need to consider interactions between the GM plant and target organisms.

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

Comments/Questions of the expert(s)

Comment 1

As feral populations are improbable in most of the European environments, there is no need to consider interactions between the GM plant and target organisms.

D.9.6 Effects on human health

Comments/Questions of the expert(s)

Comment 1

Potential herbicide residues should have been taken into account in the assessment.

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1

A feeding experiment with broilers, lasting 49 days, including from ±50% maize in the starting period to more than 60% in the finishing period, showed low mortality rates.

Comment 2

No remarks on the reported broiler trial.

Comment 3

Comment same as section D.9.6.

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

Comment 1

No comment

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

Comments/Questions of the expert(s)

Comment 1

No comment

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

Comment 1

No comment

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

Comment 1

No comment

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

Comment 1

No comment

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

Comment 1

No comment

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

Comments/Questions of the expert(s)

Comment 1

More attention should be paid on feral maize plants in southern parts of Europe. Monitoring and plant eradication measures would be taken along road sides and around storing commodities.

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

Comment 1

No comment

References

EPA (2007). Biopesticides registration action document, US Environmental Protection Agency, Washington D.C., 106 pp.

Brunet Y. (2006). Dispersion du pollen de maïs à longue distance : sources, transport, dépôt. Premier séminaire de restitution du programme ANR-OGM. Organismes génétiquement modifiés : aspects socio-économiques, alimentaires et environnementaux, 14 & 15 décembre, 2006, Paris, France, 61-64.

Hilger C, Thill L, Grigioni F, Lehners C, Falagiani P, Ferrara A, Romano C, Stevens W, Hentges F. (2004) IgE antibodies of fish allergic patients cross-react with frog parvalbumin. *Allergy*, 59, 653-60

Luna V.S., Figueroa M.J., Baltazar M.B., Gomez L.R., Townsend R., Schoper J.B. (2001). Maize pollen longevity and distance isolation requirements for effective pollen control. *Crop Sci.* 41: 1551-1557.

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

Pline-Srnic W. (2005). Technical performance of some commercial glyphosate resistant crops. *Pest Management Science* 61 (3), 225-234.

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*, Sep 4. (Epub ahead of print)

Thomas W E, Pline-Srnic W A, Thomas J F, Edmistein K L, Wells R, Wilcut J W (2004) Glyphosate negatively affects pollen viability but not pollination and seed set in glyphosate-resistant corn. *Weed science* 52 (5), 725-734.

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.

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30-08-2010

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Compilation of comments of experts in charge of evaluating the additional information received for application EFSA/GMO/UK/2008/56

Coordinator: Prof. dr. ir. Dirk Reheul
Experts: Pascal Cadot (Consultant), Peter Smet (Consultant), Hadewijch Vanhooren (KUL)
Domains of expertise of experts involved: Toxicology, immunology, alimentary allergology

INTRODUCTION

Dossier **EFSA/GMO/UK/2008/56** concerns an application of the company **Syngenta seeds** for the marketing of the genetically modified **maize Bt11xMIR604xGA21** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 19 August 2008. On the same date EFSA started the formal three-month consultation period of the Member States.
Within the framework of this consultation eight Belgian experts formulated a number of comments to the dossier. See document BAC_2008_840 for an overview of all the comments and for the list of comments actually placed on the EFSAnet on 13 November 2008.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 29 April 2010 (The EFSA Journal, 2010, 8 (5):1616)1, and published together with the responses from the EFSA GMO Panel to comments submitted by the experts during the three-month consultation period.
On 20 May 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, all the complementary information sent by the company to EFSA after 13 November 2008 was provided to the experts who evaluated the toxicological and allergenic aspects of this GM maize. They were asked to check if the new data answer the questions/comments they formulated in 2008 and, in the case the questions remain unsolved, to consider if it has an impact on the safety of this GM maize.

[1] See: http://www.efsa.europa.eu/en/scdocs/scdoc/1616.htm

List of comments received from the experts

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

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

In the meantime the MIR604 event has been evaluated by the EFSA GMO Panel (EFSA Journal 2009, 1193, 1-26), and has been approved by the Commission Decision 2009/866/EC for food and feed uses, import and processing. There is no further need that the application of the stacked event Bt11xMIR604xGA21 maize should be supplemented with the comprehensive evaluation of the MIR604 event.

Comment 2

I checked the additional information which was provided by the company. At the moment I have no further remarks concerning these dossiers.
As no information was provided concerning degradation of the Cry1Ab protein, the PAT protein and the PMI protein in simulated intestinal fluid, my previous remarks are still valid.

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

See the preceding comment concerning the MIR604 event.
Additional information was provided by the applicant as requested by the EFSA GMO panel: An updated bioinformatic analysis comparing the amino acid sequenced of the five transgenic proteins with the sequences of known toxic and general proteins using an updated database (BLASTP analysis of the NCBI Entrez Protein Database (NCBI 2009). No further comments.

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

In the meantime the MIR604 event was approved. Whole-food toxicity testing has been performed consisting of a 90-days feeding study in rats (or cattle: Bt11) and a broiler chicken study for **all three**

approved single GM maize events. In addition, a 49-days broiler feeding study was provided for the stacked event. This report was supplemented with a presentation of the data on feed intake, including cumulative feed intake, along with a statistical analysis. Moreover, the compositional analysis of the stacked event was supplemented with individual location statistical analysis data and across locations data ranges (as requested by EFSA).
Although no additional 90-days feeding study in the rat was provided for the stacked event, we are of the opinion that the whole of data available complemented with the additional provided information confirms that interactions between the single events that might impact on food /feed safety are unlikely and that the stacked event Bt11xMIR604xGA21 maize is as safe as its conventional counterpart. The deficiency of the 90-days feeding study in rats has no further impact on the safety of the GM maize.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

From an allergenicity point of view, my main concern in these files was the allergenicity of PMI in MIR604 maize.
The applicant provided the EFSA with a lot of additional information concerning the potential allergenicity of PMI. Besides the useless “proteolytic digestion test” and “thermostability test”, all analyses were performed *in silico*.
In these in silico studies, sequence homology of PMI with Hev b 13 was confirmed. Homology with Ara h 1 was also mentioned. Further in silico analysis (sequence comparison and 3D-structure comparison) concluded on the non-allergenicity of PMI.

However, I still think that the best way to rule out allergenicity in this case is to perform skin testing with PMI on subjects allergic to Hev b13. Alternatively, Western blotting (or equivalent) with sera from Hev b 13 allergic subjects would also be valuable, instead of pages of discussion based on artificially determined limits of positivity and artificially determined 3D structures. It is OK to use modelling when no other way is possible, which is not the case here.
Therefore, my question on potential allergenicity of PMI is not answered, strictly speaking. Even if this does probably not represent a major threat (due to the low levels of expression, for example), simple experiments would allow EFSA to have clear-cut results on PMI allergenicity.

My second concern was about the testing of the overall allergenicity of the transgene plant. This is still not answered, but this was also not asked by EFSA. EFSA has never supported such testing on the basis that maize is not a major allergenic food (not in the “official” list of food allergens for labelling, I suppose). This is true at the moment being, but I still think that the role of such GMO evaluation is also to avoid that maize (or anything else) BECOMES an allergenic threat. For this reason, and even if the risk of higher allergenicity in the GMO is minor, I am still in favour of testing the allergenicity of the whole transgene plant.