Bioveiligheidsraad
Conseil de Biosécurité

05-10-2010

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2010_0956

Title: Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/UK/2007/50 from Syngenta under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/UK/2007/50 was submitted by Syngenta on 14 November 2007 for the marketing (import and processing) of the insect resistant and glufosinate-tolerant genetically modified Bt11 x MIR604 maize for food and feed uses under Regulation (EC) No. 1829/2003[1].

The application was officially acknowledged by EFSA on 11 March 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). Nine 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 6 June 2008.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 29 April 2010 (The EFSA Journal, 2010, 8 (5):1614)[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 6 June 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/1614.htm>

Wetenschappelijk Instituut Volksgezondheid | Institut Scientifique de Santé Publique
Dienst Bioveiligheid en Biotechnologie | Service Biosécurité et Biotechnologie
Rue Juliette Wytsmanstraat 14 | B-1050 Brussels | Belgium
T + 32 2 642 52 11 | F + 32 2 642 52 92 | bac@wiv-isp.be | www.bio-council.be

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) and maize line Bt11 (EFSA/GMO/RX-Bt11), 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 the Biosafety Advisory Council formulated a positive advice[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.
According to the EFSA Guidance on stacked events[5], "*As long as each event in the highest number of stacked events has been risk assessed, the risk assessment of the stacked events might also be applicable to GM stacks containing fewer of these events. Thus a single risk assessment of such a stack could cover all combinations with fewer of these events",* Although the BAC may follow this statement in this context, the BAC is of the opinion that the applicant should give results of the applied material, according to the principles of good science.

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] Advice of BAC on maize line MIR604: BAC_2009_01365; Advice of BAC on maize line Bt11: BAC_2009_904
[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.
[5] Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events. *The EFSA Journal (2007) 512, 1-5*

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.

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 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.

H. 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/2007/50 and Comments submitted on the EFSAnet (ref. BAC_2008_764)
Annex II: Compilation of comments of experts in charge of evaluating the additional information received for application EFSA/GMO/UK/2007/50 (ref.BAC_2010_0830)

Bioveiligheidsraad
Conseil de Biosécurité

06 June 2008

Secretariaat
Secrétariat

N./réf. : WIV-ISP/BAC/2008_764
Email. : bac@sbb.ihe.be

Compilation of comments of experts in charge of evaluating the application EFSA/GMO/UK/2007/50
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 28 March 2008
Coordinator: Prof. Dirk Reheul
Experts: Pascal Cadot (Consultant), Rony Geers (KUL), Jean-Claude Grégoire (ULB), André Huyghebaert (UGent), Jean-Pierre Maelfait (UGent), Peter Smet (Consultant), Wim Stevens (UIA), Frank Van Breusegem (VIB), Johan Van Waes (ILVO)
Domains of expertise of experts involved: Genetics, genome analysis, genetic engineering, analysis of food/feed, industrial processing, toxicology, immunology, alimentary allergology, animal nutrition, traceability of alimentary chain, agronomy, crop protection, crop production management, herbicide tolerance, ecology, plant-insect relations, effect on non-target species, risk analysis, monitoring, nature conservation, biosafety research, maize
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

INTRODUCTION

Dossier **EFSA/GMO/UK/2007/50** concerns an application of the company **Syngenta** for the marketing of the genetically modified **maize Bt11 x MIR604** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 11 March 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). 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

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1

According to the dossier the scope of application does not include the authorization for the cultivation of Bt11 x MIR604 maize seed products in the EU in the framework of the Directive 2001/18/EC. It can however be valuable to give some remarks on the different topics, dealing with cultivation and survivability of seeds, in the case that the applicant should ask in the near future for an extension for the scope of cultivation.
So as agronomical expert I will also give some comments in this questionnaire, related to the cultivation, the agronomical value and some environmental aspects.

Comment 2

No comments

Comment 3

NB – My competence is in the environmental effects of GM plants; therefore my contribution in this dossier will be limited. Every time I will feel that the question asked is out of my field, I will use this "No comment/question" reply

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

Comments/Questions of the expert(s)

Comment 1

No comments

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Comment 1

Table 1:
- The accession number indicated for the “Maize intervening intron sequence 6 from the maize *adh*1 gene (Entrez Accession Number X04090)” is incorrect. X04090 refers to a human catalase gene.

D. INFORMATION RELATING TO THE GM PLANT

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

Comments/Questions of the expert(s)

Comment 1

No comments

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

Comments/Questions of the expert(s)

Comment 1

No comments

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1

No comments

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

Remarks concerning the survivability of seeds of maize. In the dossier it is mentioned that maize seed can only survive under a narrow range of climatic conditions. Volunteers are killed by frost. This is correct but from our experience maize seeds can survive in the soil during a not so severe winter. It can happen that out of full ears, fallen on the ground at harvest and after labouring of the land, covered with soil, some seeds survive and give plantlets during the next season. So here in the case of GMO-plants it will be necessary to have a follow up of the fields in the next year to detect for surviving plants. This information is only relevant if at a certain moment the scope would be extended to cultivation in Northern and Western Europe with moderate to cold winter conditions.

Comment SBB: Above comment is not relevant for this dossier.

Comment 2

No comments

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

Comments/Questions of the expert(s)

Comment 1

No comments

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

Comments/Questions of the expert(s)

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

D.7.1 Comparative assessment

Comments/Questions of the expert(s)

Comment 1

In this chapter it is mentioned that Bt11 x MIR604 maize was compared with relevant control maize lines that had not been genetically modified. Commercial varieties were also included in the comparison where possible. What does it mean? The Bt11 x MIR604 is tolerant to glufosinate-ammonium. So I think it is not possible to compare with commercial varieties, unless they are also tolerant to glufosinate-ammonium (= are also genetically modified).

Comment 2

Of the 56 analytes measured in grain, statistically significant differences were noted for levels of total dietary fiber (TDF) and fat, vitamin E (α-tocopherol), and linoleic fatty acid. The average values of all analytes measured for both the Bt11 x GA21 (measured in Bt11 x MIR604 x GA21) grain and the nontransgenic grain were within the ranges reported in the literature.

Comment 3

The approach is the same as for the dossier Maize MIR604 x GA21.

No remarks

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

Same as dossier 48 : Material is produced at different locations and under different environmental conditions.
No comment

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

The same compounds, as in dossier 48, are selected for analysis of maize and forage.
I have no particular comment.
I agree with the conclusion that Maize Bt11 x MIR604 is compositionally equivalent to conventional maize.

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

First of all I agree with the important remark of the applicant that measurement and observation of agronomic characteristics can add to the assessment of unintended effects of the genetic modification. The Bt11 x MIR604 maize was tested in the USA during the 2005 growing season. The results of these trials suggest that there is no statistically significant difference in grain yield or agronomic performance between the Bt11 x MIR604 maize hybrids and the corresponding near-isogenic hybrids.
So my remark: The results are only based on 1 year trials and the year effect can be given significant effects. And furthermore : were the trials treated against herbs with glufosinate ammonium so as to evaluate the real potential of the new hybrids?

Comment 2

The information received is satisfactory. In principle however, this question should be non relevant in the present application (provided there is no spillage), as the application only concerns food and feed uses for Bt11 maize.

D.7.5 Product specification

Comments/Questions of the expert(s)

Comment 1

No further remarks.

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1

No further remarks.

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

No further remarks.

D.7.8 Toxicology

Comments done under D.7.8 and D.7.8.1 summarized by the coordinator

A range of 12 - 154 μg/g dry weight of Cry1Ab protein is measured in Bt11 maize. In dossier (Bt11 x GA21) no values exceeding 36 mg/kg are shown. Is the 154 μg/g value correct?

Degradation of the Cry1Ab protein, the PAT protein, the PMI protein in simulated intestinal fluid is not mentioned. Has this test been performed? If not, why isn't it performed?

Comments/Questions of the expert(s)

Comment 1

Mean concentrations of Cry1Ab and PAT proteins are indeed comparable in both Bt11 maize and Bt11 x MIR604 maize.

a) Cry1Ab protein measured in Bt11 maize

Growth stage/ Tissue	ng/mg Tissue Dry Weight		Standard deviation
	Mean	Range	
Leaves (V9-V12)	25.88	23.89-27.63	1.35
Leaves (Anthesis)	17.82	15.99-19.74	1.54
Leaves (Seed Maturity)	16.84	12.17-19.59	3.31
Roots (V9-V12)	9.99	9.14-10.62	0.59
Roots (Anthesis)	6.41	5.67-7.72	0.79
Roots (Seed Maturity)	4.32	3.32-6.99	1.52
Kernels (Seed Maturity)	1.45	1.39-1.56	0.07
Pollen (Anthesis)[1]	0.06	0.048-0.079	0.02

[1] One pooled sample analyzed in triplicate as received (air-dried overnight). Values represent the mean of three extractions.

Question concerning the concentrations of Cry1Ab protein measured in Bt11 maize. In dossier RX-Bt11 a range of 12 - 154 µg/g dry weight is mentioned. Is the 154 µg/g value correct?

b) Cry1Ab protein measured in Bt11 x MIR604 maize

Growth stage/ Tissue	ng/mg Tissue Dry Weight		Standard deviation
	Mean	Range	
Leaves (V9-V12)	27.66	25.69-28.99	1.39
Leaves (Anthesis)	22.48	18.52-28.24	3.88
Leaves (Seed Maturity)	19.65	16.95-21.42	1.98
Roots (V9-V12)	9.97	8.49-11.43	1.31
Roots (Anthesis)	5.60	5.35-6.72	0.66
Roots (Seed Maturity)	6.21	5.29-7.05	063
Kernels (Seed Maturity)	1.74	1.44-1.93	0.20
Pollen (Anthesis)[1]	0.06	0.040-0.077	0.02

c) PAT protein measured in Bt11 maize

Growth stage/ Tissue	ng/mg Tissue Dry Weight		Standard deviation
	Mean	Range	
Leaves (V9-V12)	0.15	0.13-0.17	0.01
Leaves (Anthesis)	0.14	0.13-0.15	0.01
Leaves (Seed Maturity)	<0.05		
Roots (V9-V12)	0.17	0.15-0.20	0.02
Roots (Anthesis)	0.15	0.12-0.17	0.02
Roots (Seed Maturity)	<0.07	< 0.05-0.09	
Kernels (Seed Maturity)	<0.04		
Pollen (Anthesis)[1]	<0.024		

d) PAT protein measured in Bt11 x MIR604 maize

Growth stage/ Tissue	ng/mg Tissue Dry Weight		Standard deviation
	Mean	Range	
Leaves (V9-V12)	0.17	0.14-0.18	0.02
Leaves (Anthesis)	0.17	0.16-0.20	0.02
Leaves (Seed Maturity)	<0.05		
Roots (V9-V12)	0.15	0.12-0.19	0.03
Roots (Anthesis)	0.19	0.14-0.22	0.03
Roots (Seed Maturity)	0.08	0.05-0.10	0.02
Kernels (Seed Maturity)	<0.04		
Pollen (Anthesis)[1]	<0.024		

Mean concentrations of mCry3A and PMI proteins are indeed comparable in both MIR604 maize and Bt11 x MIR604 maize.

e) mCry3A protein measured in MIR604 maize

Growth stage/ Tissue	ng/mg Tissue Dry Weight		Standard deviation
	Mean	Range	
Leaves (V9-V12)	23.74	21.30-28.29	2.75
Leaves (Anthesis)	35.50	33.28-36.89	1.50
Leaves (Seed Maturity)	45.54	35.17-50.66	5.86
Roots (V9-V12)	17.98	16.46-20.86	1.81
Roots (Anthesis)	20.96	19.39-22.44	1.20
Roots (Seed Maturity)	21.28	14.27-25.78	4.58
Kernels (Seed Maturity)	0.68	0.61-0.78	0.06
Pollen (Anthesis)[1]	< 0.053		

f) mCry3A protein measured in Bt11 x MIR604 maize

Growth stage/ Tissue	ng/mg Tissue Dry Weight		Standard deviation
	Mean	Range	
Leaves (V9-V12)	33.44	29.71-35.97	2.61
Leaves (Anthesis)	37.96	32.88-45.06	4.98
Leaves (Seed Maturity)	46.30	32.38-69.03	14.11
Roots (V9-V12)	18.89	15.29-22.61	3.26
Roots (Anthesis)	23.87	19.55-29.50	4.89
Roots (Seed Maturity)	23.49	18.51-27.23	3.35
Kernels (Seed Maturity)	0.66	0.55-0.83	0.12
Pollen (Anthesis)[1]	< 0.053		

g) PMI protein measured in MIR604 maize

Growth stage/ Tissue	ng/mg Tissue Dry Weight		Standard deviation
	Mean	Range	
Leaves (V9-V12)	9.99	8.60-12.32	1.49
Leaves (Anthesis)	10.63	8.12-13.75	2.10
Leaves (Seed Maturity)	4.66	3.89-6.03	0.86
Roots (V9-V12)	6.03	4.95-7.70	1.06
Roots (Anthesis)	3.51	2.82-4.38	0.68
Roots (Seed Maturity)	2.58	1.56-3.54	0.71
Kernels (Seed Maturity)	1.82	1.58-2.22	0.26
Pollen (Anthesis)[1]	60.05	50.25-71.27	10.59

h) PMI protein measured in Bt11 x MIR604 maize

Growth stage/ Tissue	ng/mg Tissue Dry Weight		Standard deviation
	Mean	Range	
Leaves (V9-V12)	9.85	8.20-12.01	1.72
Leaves (Anthesis)	10.42	9.02-13.06	1.64
Leaves (Seed Maturity)	5.69	3.48-7.77	1.71
Roots (V9-V12)	5.96	5.41-6.88	0.69
Roots (Anthesis)	3.90	2.44-5.99	1.47
Roots (Seed Maturity)	2.29	1.72-2.92	0.46
Kernels (Seed Maturity)	1.85	1.38-2.72	0.53
Pollen (Anthesis)[1]	56.88	56.44-57.27	0.42

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

a) Degradation of the Cry1Ab protein in simulated gastric fluid (author).
Test was previously performed. Rapid digestion was demonstrated.

b) Degradation of the Cry1Ab protein in simulated intestinal fluid (author).
Not mentioned. Has this test been performed? If not, why isn't it performed?

c) Degradation of the PAT protein in simulated gastric fluid (author).
Test was previously performed. Rapid digestion was demonstrated.

d) Degradation of the PAT protein in simulated intestinal fluid (author).
Not mentioned. Has this test been performed? If not, why isn't it performed?

e) Degradation of the Cry3A protein in simulated gastric fluid (author).
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).
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).
Not mentioned. Has this test been performed? If not, why isn't it performed?
Comment from coordinator: see technical dossier p. 38 and comment from other expert under D.7.9

h) Degradation of the PMI protein in simulated intestinal fluid (author).
Not mentioned. Has this test been performed? If not, why isn't it performed?

i) Cry1Ab: Acute Oral Toxicity Study in Mice (author).
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.**

j) PAT: Acute Oral Toxicity Study in Mice (author).
Lack of acute toxicity was demonstrated earlier. **No further testing is needed.**

k) Cry3A: Acute Oral Toxicity Study in Mice (author).
Lack of acute toxicity was demonstrated earlier. **No further testing is needed.**

l) PMI: Acute Oral Toxicity Study in Mice (author).
Lack of acute toxicity was demonstrated earlier. **No further testing is needed.**

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

a) 42-day poultry feeding study (Brake, 2007)

Broiler chickens fed Bt11 x MIR604 Positive diets did not differ in survival, growth, or feed conversion efficiency when compared with broiler chickens fed Bt11 x MIR604 Negative diets or diets prepared with commercially available maize grain. Diets prepared with Bt11 x MIR604 transgenic maize grain supported rapid broiler chicken growth at low mortality rates and excellent feed conversion ratios without a significant impact on overall carcass yield or quality. The Bt11 x MIR604 transgenic maize grain had no observed deleterious effects on bird health in this study.

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

Not performed.
The composition of the genetically modified plant is not substancially modified, except for the inserted traits. So, at this time, **further testing is not recommended**.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

The modification implies:
- Event Bt11 maize (hereafter referred to as 'Bt11 maize') which expresses a truncated Cry1Ab protein for control of certain lepidopteran pests and a phosphinothricin acetyltransferase (PAT) protein that confers tolerance to herbicide products containing glufosinate ammonium.

- Event MIR604 maize (hereafter referred to as 'MIR604 maize') which expresses a modified Cry3A (mCry3A) protein for control of certain coleopteran pests and a phosphomannose isomerase (MIR604 PMI) protein, which acts as a selectable marker enabling transformed plant cells to utilize mannose as a primary carbon source.

The allergenicity of the proteins was assessed:

• *The sources of the transgenes were considered. None of the four proteins expressed in Bt11 x MIR604 came from donors with allergenic potential.*
• *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 mCry3A proteins show no homology to any known or putative allergenic proteins.*
• *One region of sequence homology of eight contiguous identical amino acids between MIR604 PMI and a known allergen was identified. The potential for an allergic reaction in individuals already sensitised to the allergenic protein was assessed by specific serum screening methodology. The results of the serum screening analysis demonstrated no cross-reactivity between the allergic patient's serum IgE and MIR604 PMI. Details of this assessment can be found in Part I, Appendix CBI.3 of application EFSA-GMO-UK-2005-11. 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. Details of this analysis can be found in Appendix 8.*
• *The susceptibility of Cry1Ab, PAT, mCry3A and PMI 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, mCry3A and MIR604 PMI will be readily digested as conventional dietary protein under typical mammalian gastric conditions*.

It was argued that the Hev b 13 protein is only allergenic when glycosylated and that the maize protein is not glycosylated.

The parvalbumin is a very common allergen with existing cross reactivity between parvalbumins of different species (fish, frog) (Hilger et al. 2004, Ebo and Stevens, 2001).

Because of the partial identity of both proteins extreme care has to be taken and it would be wise to test the proteins with a wide array of sera of patients allergic to natural rubber latex on one hand and fish allergy on the other hand. Follow up after the use of these proteins is mandatory and warning about the presence of these proteins with partial identity with Hev b 13 and parvalbumin seems advisable

Comment as summarized by the coordinator

Two homologies were found when testing for allergenicity:
- one with Hev b 13 (natural rubber latex, Hevea brasiliensis) and
- one with alpha parvalbumin from Rana sp.

Therefore it is :
1) highly recommended to test the reactivity of these proteins on patients with a known allergy to Hev b13 and parvalbumine 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.

The company should take care to use updated allergen databases for its searches.

Comment 2

Assessment of the allergenicity of the newly expressed proteins.
According to the data currently available, Cry1Ab, mCry3A and PAT are unlikely to be allergenic. However, the company should take care to use updated allergen databases for the searches.
The case of MIR604 PMI is more complicated. In a previous dossier, it was shown to possibly cross-react with alpha-parvalbumin from a certain *Rana* species. This, however, was rightly shown by the applicant, with patient serum testing, to be non-relevant.
In the new dossier, the applicant describes possible cross-reactivity with a moderately important latex allergen, Hev b 13. Citation of the applicant: "when using the more appropriate method of determining percent identity and taking the full alignment length into account, as supported by the FARRP database, the MIR604 PMI – Hev b13 homology is only 29.6% (29 identities/ 98aa alignment). This is not considered a significant allergen homology as per the guidelines set by the Codex Alimentarius Commission (2003)."
The reviewer agrees that sequence comparison on full alignment length is more appropriate to evaluate identity with allergens. However, conversely to what is stated in the guidelines of the Codex Alimentarius, a homology of 29.6% could be an issue, as this represents 29 identical aminoacids between the two proteins, enough to construct several cross-reactive epitopes. Therefore, it is required that the reactivity of PMI be evaluated 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 has been documented, although it is not recognized as a major allergy concern. Some maize allergens have already been described in the literature (Pastorello et al. 2003; Pasini et al. 2002, Weichel et al. 2006).
Due to the introduction of the 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 genetically modified Bt11 x MIR604 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)

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)

Comment 1

I agree that the risks (should spillage occur) are extremely low, as maize does not reproduce outside of cultivation.

Comment 2

Provided information: sufficient

Additional comment from coordinator

It is very unlikely that spillage will occur within agricultural land. Should this occur, there are, *anno* 2008, no indications that the transgene would have a selective advantage in current Belgian agricultural practices.
The germination and persistence of spilled kernels along transport ways is not very probable. Should spilled kernels germinate and flower occasionally, pollen transfer remains possible. So, according to the precautionary principle, it is recommended to monitor transport routes in order to guarantee traceability. On top of this, measures to be taken in case of accidental spillage are needed as is information regarding the packing and other means of confinement during transportation and storage.

And of course, should transgenic plants survive, they can not be killed by the herbicides they are made resistant for, so the quote of the applicant "…could be easily controlled by **any** of the current agronomic measures….." is not true.

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

Comment 1

Provided information: sufficient

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

Comment 1

There is a high probability that (spillage+establishment+contamination) will be limited at some parts of the itinerary (e g at ports), but this holds not necessarily true along the transportation routes. Even though it can not survive the winter, maize from spilled seeds can develop one generation on the sites of spilling, leading to potential dissemination of pollen. 1% of the pollen beyond 50 m (Sears and Stanley-Horn, 2000) does not seem negligible to me. If we do not know the routes, we do not know if maize is grown along the roads
More specific details are needed regarding the packing and other means of confinement during transportation and storage, as well as measures to be taken in case of accidental spillage.

Comment 2

Provided information: sufficient

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

Comment 1

Not applicable

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

Comments/Questions of the expert(s)

Comment 1

Provided information: sufficient

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)

Comment 1

The number of animals in the broiler trial is sufficient for adequate power in the statistical analysis.

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

Comment 1

Provided information: sufficient

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

Comments/Questions of the expert(s)

Comment 1

In this paragraph it is mentioned again that the scope of application does not include cultivation of maize plants of Bt11 x MIR604 maize in the EU. Nevertheless I give here some remarks in the case that the applicant should ask in the near future for an extension for the scope of cultivation. In the framework of the EU- regulation 2002/53 a new variety have to be submitted to DUS (Distinctness, Uniformity, Stability) and VCU (Value for Cultivation and Use) tests before the variety can be commercialised. The new variety has to be compared with the best existing standard varieties. So my question here is : can the GM- maize be incorporated in normal VCU trials, for example treated with specific herbicides for maize and will the agronomical value be the same as tested in trials, where herbicides for which the variety is tolerant were used?

Comment SBB: the above comment is not relevant for this dossier.

Comment 2

Not relevant here.

Comment 3

Not applicable

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

Comment 1

I agree with the comments given by the applicant.

Comment 2

Provided information: sufficient

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

Comment 1

If seeds were imported by train containers for making food and feed, some monitoring has to done to control if there are no maize plants along the railway roads. As already mentioned under a moderate winter seeds of maize can survive and can give plantlets in the next spring; so these plants have to destroyed.

Comment 2

We support the recommendation of ACRE (2006) that provision of detailed arrangements for general surveillance post-market monitoring plans for the import and processing of grain from GM maize should be made a condition of any consent.
Monitoring and reporting on the possible establishment of feral populations should be a point of particular attention in the report to be delivered annually to the Commission. More details on the organisation and implementation of that monitoring would be useful.

Comments summarized by the coordinator

As already mentioned in D.9.1 it is recommended to record all transport routes in order to guarantee traceability. So we support the recommendation of ACRE (2006) that provision of detailed arrangements for general surveillance post-market monitoring plans for the import and processing of grain from GM maize should be made a condition of any consent.

Monitoring and reporting on the possible establishment of feral populations should be a point of particular attention in the report to be delivered annually to the Commission. More details on the organisation and implementation of that monitoring would be useful.

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)

Comment 1

The proposed general surveillance of the impact of the GM plant and the provisions concerning traceability and labelling satisfy.

Comment 2

The essential elements of the surveillance plan for Bt11 x MIR604 maize appear vague. For example (Technical dossier p. 49, but see also Appendix 11):
" *i. The best possible chance of detecting an unanticipated adverse effect would be ensured by having an adequate number of people, with relevant experience, involved in the surveillance process. It follows, therefore, that those persons or organizations normally involved in the import and use of maize will be in the best position to participate in a general surveillance plan.*
ii. In order to allow detection of the broadest possible scope of unanticipated adverse effects it is proposed that general surveillance is performed by selected, existing networks, in combination with a common industry approach…"

Representative organisations have been identified among the importers, grains handlers and processors. However, the initiative and responsibility lie exclusively on these organisations, as illustrated by the "*Suggested questions to be asked as part of the General Surveillance Plan*" (p. 10 of Appendix 11)

Additional comment from the coordinator

If (one of) these components of the monitoring network fail to do their share of the work, the whole monitoring network is at risk.
Therefore a strong and solid monitoring plan is necessary.

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

References

ACRE (2006). General advice on notifications for import and marketing of GM maize grain. (http://www.defra.gov.uk/environment/acre/advice/pdf/acre_advice74.pdf)

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

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, 653-660

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

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

Bioveiligheidsraad
Conseil de Biosécurité

30-08-2010

Secretariaat
Secrétariat

N./réf. : WIV-ISP/BAC_2010_0830
Email. : bac@wiv-isp.be

Compilation of comments of experts in charge of evaluating the additional information received for application EFSA/GMO/UK/2007/50

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

INTRODUCTION

Dossier **EFSA/GMO/UK/2007/50** concerns an application of the company **Syngenta** for the marketing of the genetically modified **maize Bt11 x MIR604** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 11 March 2008.
Within the framework of this consultation eight Belgian experts formulated a number of comments to the dossier. See document BAC_2008_764 for an overview of all the comments and for the list of comments actually placed on the EFSAnet on 6 June 2008.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 29 April 2010 (The EFSA Journal, 2010, 8 (5):1614)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 6 June 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/1614.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

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.7.9 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.