Bioveiligheidsraad
Conseil de Biosécurité

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Secretariaat
Secrétariat

O./ref.: WIV-ISP/BAC/2009_904

Title: Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/RX-Bt11 from Syngenta under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/RX-Bt11 was submitted by Syngenta on 29 June 2007 for the renewal of authorisation of the insect resistant genetically modified (GM) maize Bt11 for food and feed applications according to Article 8 and 20 of Regulation (EC) No. 1829/2003[1].

Bt11 maize has already been subject previously to several notifications:
- For import and use of grain in the European Union according to Directive 90/220/EEC. Approved by Commission Decision 98/292/EC[2].
- For the placing on the market as a novel food or novel food ingredient under Regulation (EC) No 258/97. Approved by Commission Decision 2004/657/EC[3].
- For the placing on the market for cultivation, feed and industrial processing (notification C/F/96.05.10 submitted under Directive 2001/18/EC); the authorization procedure is still running. Belgium has previously issued a scientific opinion related to this notification (report of 8 August 2006 of the Division of Biosafety and Biotechnology on mandate of the Biosafety Advisory Council).

Additionally, Bt11 maize has been entered on the community register of GM Food and Feed as an existing product under Article 8 and 20 of Regulation (EC) No 1829/2003.

The application EFSA/GMO/RX-Bt11 was officially acknowledged by EFSA on 17 March 2008. On the same date EFSA started the formal three-month consultation 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 chosen from the common list of experts drawn up by the Biosafety Advisory Council and the

[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] Commission Decision 98/292/EC of 22 April 1998 concerning the placing on the market of genetically modified maize (Zea mays L. line Bt-11), pursuant to Council Directive 90/220/EEC.(OJ L 131, 5.5.1998, p.28)
[3] Commission Decision 2004/657/EC of 19 May 2004 authorising the placing on the market of sweet corn from genetically modified maize line Bt11 as a novel food or novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council (OJ L 300, 25.09.2004, p.48)

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Division of Biosafety and Biotechnology (SBB) to evaluate the dossier. 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 10 June 2008.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 28 January 2009 (The EFSA Journal, 2009, 977, 1-13)[4], and published together with the responses of the EFSA GMO Panel to comments submitted by the experts during the three-month consultation period.

On 18 February 2009 the opinion of EFSA was forwarded to the Belgian experts. They were invited to give comments and to react if needed to the answers given by the EFSA GMO Panel, in particular in case the comments formulated in their initial assessment of the dossier were not taken into account in the opinion of EFSA.

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

Scientific evaluation

1. Environmental risk assessment

According to the Biosafety Advisory Council no major risks were identified concerning the environment[5].
The experts noticed however that the proposed post-marketing monitoring plan was weak and that the essential elements of the surveillance plan for Bt11 maize appeared vague.
On request of EFSA the applicant submitted in August 2008 an updated monitoring plan which includes the requirement of reporting the indirect and delayed effects on a yearly basis. The new plan is based on the Industry Harmonised Monitoring Plan. The additional information provided has been considered satisfactory.

2. Molecular characterisation

With regard to the molecular characterisation, the Belgian experts are of the opinion that information received is sufficient.

3. Food/feed safety assessment

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

3.2. Following the comments submitted by the Belgian experts, the Biosafety Advisory Council recommended that for the assessment of allergenicity of the newly expressed

[4] See: < http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902337160.htm>
[5] As the application doesn't imply a cultivation of the GM crop in the EU, a full environmental assessment is not required in EFSA procedure and was not achieved.
[6] OECD, 2001. Consensus Document on Compositional Considerations for New Varieties of Soybean: Key Food and Feed Nutrients and Anti-Nutrients. ENV/JM/MONO(2001)15.
http://www.olis.oecd.org/olis/2001doc.nsf/c5ce8ffa41835d64c125685d005300b0/

proteins new bioinformatic studies should be performed using updated databases. On request of EFSA the applicant performed those studies and the results were considered satisfactory.

3.3. The Biosafety Advisory Council observes that the allergenicity of the whole GM maize has not been evaluated. The introduction of the transforming DNA might interfere with the expression levels of other maize proteins, including allergens. Therefore, it might be relevant to analyze whether the expression levels of allergens is increased and to carry out IgE binding studies.

4. Nutritional value

In the study with laying hens 60 instead of 10 animals per treatment would have provided the right power in the statistical analysis, based on the reported standard deviation and differences between mean values.
In the study of the lactating dairy cows the number of animals per treatment are sufficient for testing the somatic cell count, but not for testing milk production.

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 notifier to the EFSA GMO Panel questions and considering the data presently available, the Biosafety Advisory Council,

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

In addition, the Biosafety Advisory Council recommends:
1) To include the analysis of dietary fibre in the compositional analysis of food and to adapt the OECD consensus documents accordingly;
2) To consider introducing assessment of allergenicity of the whole GM crops in the frame of the revision of the EFSA guidance document on Food/Feed safety assessment;
3) To preset the sample size in feeding trials in order to be able to draw statistically sound conclusions (for instance no statistical sound appreciation of the nutritional value could be made from the trial with laying hens).

p.o.

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

Annex: Full comments of experts in charge of evaluating application EFSA/GMO/RX-Bt11 *and comments submitted on the EFSAnet (ref: BAC_2008_766)*

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Conseil de Biosécurité

10/06/2008

Secretariaat
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Compilation of comments of experts in charge of evaluating the application EFSA/GMO/RX-Bt11
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/RX-Bt11** concerns an application of the company **Syngenta** for the renewal of authorisation of the genetically modified **maize Bt11** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 16 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 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

No comments

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: the above comment is not relevant for this dossier.

Comment 2

No comments

Comment 3

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.

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 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 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

The biochemical composition of kernels produced by Bt11 maize (sweet and field maize) has been analyzed and compared to the biochemical composition of kernels produced by isogenic non modified

maize. No statistically significant differences which could be attributed to the genetic modification were found.

Comment 3

Bt11 maize is compared with isogenic non-transgenic maize.
No remarks.

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

Maize kernels and forage, of Bt11 maize, have been produced at different locations in the US and the EU.
Analysis results are compared with results from control maize grown under the same conditions.
Mean values are also compared with literature data.
This is a traditional approach.
No remarks.

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

Nutrients were selected according to the OECD recommendations.

The range of nutrients analyzed is somewhat different for the EU and the US maize kernel samples. In addition to proximate analysis other constituents were analyzed in one or both set of samples. Information is available about amino acids and fatty acids profile, minerals such as copper magnesium, manganese and zinc, vitamins such as B1, B2, folic acid, and niacin, xanthofyll, trypsin inhibitor and phytic acid.

Results for forage include proximate analysis, including fibre according to the “feed “ approach, a broad range of minerals, including major minerals, and specific feed parameters.
The applicant concludes that Bt11 maize is substantially equivalent to conventional maize.

Comments:
Information on nutrients and anti-nutrients is less comprehensive in comparison to other recent maize dossiers.
Information on niacin (B3 or PP) an important vitamin in maize is included.
Anti-nutrients and secondary plant metabolites are only partly covered.
Xanthofyll has been studied; there is increased interest in this compound in relation to human health.

As for other maize dossiers there is no information on resistance to moulds, particularly in relation to mycotoxin formation. Some information must be available taking into account the history of this dossier.

Nevertheless I agree with the conclusion that Bt11 maize is nutritionally equivalent to conventional maize

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

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 maize was tested in the USA during the 1995 growing season. The results of these trials suggest that there is no statistically significant difference in grain yield or agronomic performance between the Bt11 maize line and the corresponding near-isogenic 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

The applicant concludes that, taking onto account substantial equivalence, Bt11 maize is as safe and wholesome as conventional maize;
No further comment.

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1

Bt11 maize will be processed in the same way as conventional maize. No particular effects are to be expected.

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

No anticipated changes are to be expected, due to the introduction of Bt11 maize.
No further comments.

D.7.8 Toxicology

Summary of comments done under D.7.8 and D.7.8.1 done 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 in simulated intestinal fluid.
Not mentioned. Has this test been performed? If not, why wasn't this done?

Degradation of the PAT protein in simulated intestinal fluid.
Not mentioned. Has this test been performed? If not, why wasn't this done**?**

Comments/Questions of the expert(s)

Comment 1

Question concerning the concentrations of Cry1Ab protein measured in Bt11 maize.
A range of 12 - 154 μg/g dry weight is mentioned. Otherwise, in dossier 49 (Bt11 x GA21) the following values for Cry1Ab protein concentrations in Bt11 maize can be found:

Growth stage/ Tissue	ng/mg Tissue Dry Weight		Standard deviation
	Mean	Range	
Leaves (V9-V12)	33.81	29.44-38.07	3.21
Leaves (Anthesis)	35.81	28.07-46.70	6.97
Leaves (Seed Maturity)	10.75	9.92-12.44	1.02
Roots (V9-V12)	13.90	11.90-15.81	1.51
Roots (Anthesis)	9.47	8.59-10.15	0.61
Roots (Seed Maturity)	4.66	4.27-5.20	0.37
Kernels (Seed Maturity)	1.24	0.84—1.60	0.32
Pollen (Anthesis)[1]	0.10		

Is the 154 µg/g value correct?

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

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

c) Degradation of the PAT protein in simulated gastric fluid.
Rapid digestion was demonstrated previously. **No further testing is needed.**

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

e) Cry1Ab: Acute Oral Toxicity Study in Mice (Finlay, 2006).
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.**

f) PAT: Acute Oral Toxicity Study.
Lack of acute toxicity was demonstrated earlier. **No further testing is needed.**

Phosphinothricin Acetyltransferase Protein (PAT): Assessment of Amino Acid Sequence Homology with Known Toxins.
To determine if phosphinothricin acetyltransferase protein as expressed in Event Bt11 maize (corn) plants has any significant amino acid sequence homology to known toxins, a search was performed using the BLASTP search program. Using conservative search criteria, it was concluded that the PAT query sequence showed no significant sequence homology to any proteins identified as, or known to be, toxins.

Cry1Ab as Expressed in Event Bt11 Maize: Assessment of Amino Acid Sequence Homology with Known Toxins.
To determine if the Cry1Ab protein as expressed in Event Bt11 maize (corn) plants has any significant amino acid sequence homology to known toxins, a search was performed using the BLASTP search program. Using conservative search criteria, 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.

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

In the study with laying hens 60 instead of 10 animals per treatment would have provided the right power in the statistical analysis, based on the reported standard deviation and differences between mean values.
In the study of the lactating dairy cows the number of animals per treatment are sufficient for testing the somatic cell account, but not for testing milk production.

Comment 2

(a) 14-day feeding study on laying hens.
Laying hens with high egg production were fed diets containing Bt11 maize or non-transgenic counterpart during 14 days. **No effect of the diet was observed upon survivability, health, egg production or egg weights. In addition, no residue of Cry1Ab and PAT protein was detected in eggs nor in animal tissues.**

(b)Evaluation of Bt11 maize in broiler chickens (42-day feeding study).
Rapidly growing broilers have been used for nutritional testing of Bt11 maize (Brake et al., 2003). The animals received one of four diets containing kernels derived from Bt11 maize (treated and non-treated with glufosinate ammonium), a non-transgenic control line, and a commercial reference line. Diets were amended so that the metabolisable energy and crude protein content were similar. Growth, mortality, feed conversion ratio and carcass yield at 48 days were similar in the chickens fed with the different diets. **In conclusion, no consistent effects of the intake of Bt11 maize on the performance of chicken broilers have been observed**.

(c) 14-day feeding study in high producing dairy cows.
Three groups of 4 dairy cows were fed fresh chopped whole plant maize (ca. 22.7 kg of dry matter per animal and per day) of either Bt11 maize, another insect tolerant transgenic maize (Bt 176) and the non-transgenic, near isogenic counterpart of event 176. Both Bt11 maize and Bt 176 have been modified with the Cry1Ab and PAT proteins. Bt 176 derived from plants contained intermediate levels,

and plants from the Bt11 maize variety contained relatively high levels of Cry1Ab protein. **Milk production, feed intake, milk composition, and udder health were similar for all study groups. Cry1Ab and PAT proteins could not be detected in milk of cows fed the genetically modified maize lines.**

(d) Utilization of Bt maize residues by grazing beef steers and Bt maize silage and grain by growing beef cattle and lactating dairy cows has been reported by Folmer et al. (2002). Sixteen lactating dairy cows received diets containing silage of an early- and late-maturing variety of Bt11 maize or a control with the corresponding non-transgenic near isogenic maize line during 21-day feeding periods. No differences were observed between Bt11 maize and control maize for feed intake, body weight, milk production, and milk composition (lactose, protein, fat), as well as ruminal pH and volatile fatty acids. In addition no effects were observed of the transgenic trait on in situ ruminal digestion of neutral detergent fibre of maize.

(e) The same silages as those used for the dairy cow study were used in a beef cattle study which lasted for 101 days. Measurements included feed intake and body weight. Dry matter intake was significantly higher in steers fed early- and late-maturing Bt11 maize when compared with those fed diets containing non-GM silage. In addition, average daily weight gain in early maturing Bt11 maize-fed steers was higher than in control-fed steers, while final body weight and feed efficiency was decreased in steers fed late maturing Bt11 maize compared with steers fed control maize. In conclusion, the slightly higher dry matter intake was not associated with other effects on performance of beef cattle fed Bt11 maize that would be consistent for diets of both Bt11 maize lines.

f) 90-Day rat feeding study (author).
Not included.

Conclusion: At this moment, no further testing of whole food/feed is needed.

A review of peer reviewed scientific data on the GMO and derived food and feed which may be relevant for the safety of the GM product for humans and animals and for the environment that have become available since the original authorisation.
The literature review indicates that performance, health, and nutrient use by farm animals are similar when fed either conventional or Bt11 maize-derived crops, and/or its coproducts. Furthermore, no biologically relevant differences in the composition of animal products, including meat, milk, and eggs, have been reported between farm animals fed diets containing commercially available, biotechnology-derived crops and those fed diets containing conventional genetic counterparts.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

The allergenicity of the GM plant was evaluated using different approaches:

1. The source plant maize was evaluated as not having an associated allergenicity
2. The Cry1Ab protein was evaluated as having no allergenicity:

The Bt-toxin expressed in Bt11 maize, though in truncated form, was found to be equivalent to that occurring naturally, and equivalent to that produced for use as the biopesticide that is widely used by the organic food industry.
The Cry1Ab protein produced by Bt-11 corn was demonstrated to be equivalent to the microbially-produced protein in terms of the N-terminal sequence, immunoreactivity and post-translational modification. The microbially-produced protein is considered to be a suitable substitute for plant-expressed Cry1Ab for allergenicity studies.
3. The Cry1Ab amino acid sequence (615 amino acids) was systematically compared to the Syngenta Biotechnology, Inc. (SBI) Allergen Database. This database contains the amino acid sequences of known and putative protein allergens, including gliadins, and was initially compiled from entries in the following database sources:
4. Two different searches were performed comparing the amino acid sequence of PAT to the sequences of entries in the SBI Allergen Database. First, overall sequence homology was examined by comparing sequential 80-amino acid peptides of the Cry1Ab amino acid sequence to the allergen sequences using the FASTA search algorithm (Pearson and Lipman, 1988).1 Each successive 'window' of 80 amino acids was offset from the previous window by one residue, such that each peptide overlapped the previous peptide by 79 amino acids. (For example, the first peptide contained amino acid residues 1 – 80, the second contained amino acid residues 2 –81, etc.). Any 80-amino acid peptide of the query sequence having greater than 35% amino acid identity to an allergen sequence was defined as having significant homology to the allergen sequence (FAO/WHO, 2001).
In the second search, the Cry1Ab amino acid sequence was screened for matches of eight or more contiguous amino acids (Hileman *et al.*, 2002) using a program, developed by Syngenta, that compares every possible peptide of eight contiguous amino acids between the query sequence and the allergen sequences in the SBI Allergen Database. The purpose of this analysis was to screen for short, local regions of amino acid identity that might indicate the presence of common IgE-binding epitopes.
The results of these analyses revealed no significant sequence homology between any sequential Cry1Ab 80-amino acid peptide and any entry in the SBI Allergen Database. Additionally, there were no alignments of eight or more contiguous identical amino acids between Cry1Ab and any of the proteins in the SBI Allergen Database.. In conclusion Cry1Ab shows no significant amino acid sequence homology to known or putative allergenic proteins.
5. Rapid and extensive degradation by pepsin was evaluatd; this evaluation is relative since it is known that some proteins as e.g. the Mal d 1 allergen can induce symptoms in an undigested form. Other plant allergens (e.g. from carrot and potato) have similar potentential.
6. The PAT allergen was also evaluated for sequence homology. To update the assessment if the amino acid sequence of the phosphinothricin acetyltransferase protein (PAT) expressed in Bt11 maize has any significant homology to known allergens, two different searches were performed comparing the PAT amino acid sequence to the sequences in the SBI Allergen Database.
The PAT amino acid sequence (183 amino acids; Figure 1; Entrez Database Accession No. AAU00088 (NCBI, 2006)) was systematically compared to the Syngenta Biotechnology, Inc. (SBI) Allergen Database.
7. As for the Cry1Ab protein pepsin digestibility was evaluated. Here the same remarks as for the Cry1Ab.

Conclusion:
There are no data indicating allergenicity of the protein involved to date. Since allergy is an individual trait follow up has to be continued.

Comment 2

Assessment of the allergenicity of the newly expressed proteins.

Agreed with the statement that, with the current knowledge, Cry1Ab and PAT are unlikely to be allergenic. However, the allergen databases that have been used to construct the company internal allergen database for sequence comparisons should be updated (as the major ones dates back from 2001).

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 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.

Above comments as summarized by the coordinator

With the current knowledge, Cry1Ab and PAT are unlikely to be allergenic. However, the allergen databases that have been used to construct the company internal allergen database for sequence comparisons should be updated (as the major ones dates back from 2001).

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 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.

Since allergy is an individual trait, follow up has to be continued.

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

Provided information: sufficient

Comment 1

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

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

Provided information: sufficient

Comment 2

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.

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)

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 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 applicable

Comment 3

Non relevant here.

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 the monitoring are necessary.

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 maize appear vague. For example (Technical dossier p. 55, but see also Appendix 10.3):
"*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*"
"*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*…"

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*" (Appendix 10.3, p. 11), e.g.: "*Have you informed your member associations who represent importing, merchanting and handling companies to ask their own member companies to monitor…?*".
If one of these components of the monitoring network does not fully comply or provides inadequate information, the whole monitoring network is at risk.

Last sentence as rephrased by 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)

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