Bioveiligheidsraad
Conseil de Biosécurité

18-06-2010

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2010_0531

Title: Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/RX-MON863 from Monsanto under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/RX-MON863 was submitted by Monsanto on 29 June 2007 for renewal of authorisation of the insect resistant genetically modified (GM) maize MON863 for food and feed purposes (feed, feed materials, and food and feed additives) according to Articles 8 and 20 of Regulation (EC) No. 1829/2003[1].

Maize MON863 was lawfully placed on the market as feed and feed materials produced from maize MON863 and as food and feed additives before the date of application of Regulation (EC) No. 1829/2003.

Maize MON863 and stacked event MON863 × MON810 was also subject previously to a notification for import and processing (notification C/DE/02/9 submitted under Directive 2001/18/EC) and approved by Commission Decision (2005/608/EC) of 8 August 2005[2]. In the frame of the evaluation of this notification Belgium has previously issued 1 scientific opinion related to MON863.
Moreover Maize MON863 was also subjected to a notification as novel food and novel food ingredients submitted under Regulation (EC) No 258/97 and approved by Commission decision (2006/68/EC) of 13 January 2006[3].

Additionally, Maize MON863 has been entered on the community register of GM food and feed[4].

The application EFSA/GMO/RX-MON863 was officially acknowledged by EFSA on 5 June 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

[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 of 8 August 2005 concerning the placing on the market, in accordance with Directive 2001/18/EC of the European Parliament and of the Council, of a maize product (Zea mays L., line MON 863) genetically modified for resistance to corn rootworm.
[3] Commission Decision of 13 January 2006 authorising the placing on the market of foods and food ingredients derived from genetically modified maize line MON 863 as novel foods or novel food ingredients under Regulation (EC) No 258/97 of the European Parliament and of the Council (notified under document number C(2005) 5939) (OJL, 34, 07.02.2006, p.26)
[4] see: http://ec.europa.eu/food/dyna/gm_register/index_en.cfm

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 Division of Biosafety and Biotechnology to evaluate the dossier. Five experts answered positively to this request and formulated a number of comments on the dossier, which were edited by the coordinator. See Annex 2 for an overview of all the comments and for the list of comments actually placed on the EFSAnet on 5 September 2008.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 10 March 2010 (The EFSA Journal, 2010, 8 (03):1562[5]), and published together with the responses of the EFSA GMO Panel to comments submitted by the experts during the three-month consultation period.

On 31 March 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.

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

The scope of this application is feed (feed materials and feed additives) and food additives which are produced from GM maize MON863 and only includes products which contain no viable plant parts. Therefore, there are no requirements to perform an environmental risk assessment in the context of this specific application. Such an assessment has already been performed in the frame of notification C/DE/02/9 submitted under Directive 2001/18/EC.

2. Molecular characterisation

With regard to the molecular characterisation, on request from the GMO panel of EFSA the applicant submitted complementary information which was not reviewed by the Belgian experts.

3. Food and feed safety assessment and nutritional value

3.1. Assessment of compositional analysis
The Biosafety Advisory Council is of the opinion that the information provided on the composition of the GM maize does not raise any safety concerns.

3.2. Assessment of toxicity
In previous evaluations (see context) no major risks were identified concerning toxicity.
The Biosafety Advisory Council concludes that this statement continues to be valid.

[5] See: http://www.efsa.europa.eu/en/scdocs/scdoc/1562.htm

3.3. Assessment of allergenicity
Maize is not a major allergen source. The potential allergenicity of the newly introduced proteins has been assessed. No allergenicity assessment was performed on the whole GM maize. With regard to allergenicity, the Biosafety Advisory Council is of the opinion that the information provided is sufficient and does not raise safety concerns.

3.4. Nutritional value
In previous evaluations (see context) no major risks were identified concerning nutritional value. The Biosafety Advisory Council concludes that this statement continues to be valid.

4. Monitoring

As the allergenicity of the whole GM maize has not been assessed, it is recommended to take up monitoring of allergenicity as part of the general surveillance. As the statistical power of the animal trials is not perfect, the Biosafety Advisory Council advises to be vigilant on the chronic toxicity and nutritional value.

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 human and animal health were identified.

In addition, the Biosafety Advisory Council recommends following up any unanticipated allergenicity aspects of the GM maize in the existing allergenicity monitoring systems and advises to be vigilant on the chronic toxicity and nutritional value.

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

Annex I: Minority declarations of Lucette Flandroy and Damien Winandy, members of the Biosafety Council
Annex II: Full comments of experts in charge of evaluating application EFSA/GMO/RX-MON863 *and comments submitted on the EFSAnet (ref: BAC_2008_802)*

Annex I
Minority declarations

Minority Declaration of Lucette Flandroy (21/06/2010):

I cannot agree with the final conclusion of the BAC advice proposed on that file during the meeting of the BAC on this Friday 18 June 2010. I indeed consider the conclusion and the synthesis of the scientific evaluation expressed in this advice as being insufficiently informative about the reality of data for the competent Ministers to take a decision on the basis of scientific knowledge.
I favored indeed another formula as a conclusion, that had also been proposed during the discussions of the BAC on this file: " ……**the BAC : Can not draw conclusions about the safety of this GM maize due to the lack of quality of animal trials for testing the toxicity and the nutritional value. "**

The number of animal replications is indeed too low, says one of our BE experts, to allow statistical sound conclusions. (see Compilation of comments p.7, 8 and 12,13)
I consider that this situation is unacceptable especially for the renewal of a GMO file that has been subject of various and prolonged safety controversies (a.o. relatively to signs of potential hepato-renal, hematological and endocrinal toxicity) in the context of its 1st approval .(In addition, this last was obtained under a previous less stringent regulation). My opinion is that it was the duty of the applicant to take the opportunity of this application renewal to quiet all the safety doubts linked to those uncertainties by making new and extensive statistically adequate tests; this has not been done.
Between the 1st and the 2nd application, some articles published in the scientific literature conclude that the consumption of MON863 results in no adverse effects on some biological parameters in calves or pigs. Those studies however do not consider extensively the various biological parameters that have brought safety concerns .
In addition, no epidemiological study has been performed and no appropriate monitoring system is presently working at the EU level that would allow to draw first general safety conclusions after some years of this GMO on the market. Post-marketing monitoring should in any case not be a substitute for pre-approval risk assessments that can be done with the present state of the art.

In other words, if the risk evaluation tests made for this application indeed do not suggest any short- or mid-term major heath negative impact, it is impossible from the tests performed to exclude mid-term less major and thus potential long-term (chronic) major negative impacts.

It is the responsibility of the competent ministers to approve or not this GMO application in this context of scientific incertitude. In the absence of any more convincing tests results, I would, from a scientific point of view, give a negative advice on this application.

Minority Declaration of Damien Winandy (22/06/2010):

As member of the Belgian Biosafety Advisory Council I cannot agree with the Council's advice. My opinion is that the Council cannot conclude on the safety of this GM maize as feed, due to the lack of scientific quality of the animal trials intending to test its toxicity and its nutritional value.

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05/09/2008

Secretariaat
Secrétariat

N./réf. : WIV-ISP/BAC_2008_802
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Compilation of comments of experts in charge of evaluating the application EFSA/GMO/RX-MON863
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 26 June 2008
Coordinator: Prof. dr. ir. Dirk Reheul
Experts: Jacques Dommes (ULg), Armand Christophe (UGent), Rony Geers (KUL), Peter Smet (Consultant), Wim Stevens (UIA)
Domains of expertise of experts involved: Genetics, molecular characterisation, genetic engineering, transgene expression, human nutrition, animal nutrition, substantial equivalence of alimentary products, statistics, toxicology, immunology, alimentary allergology
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

INTRODUCTION

Dossier **EFSA/GMO/RX-MON863** concerns an application of the company **Monsanto** for the renewal of marketing authorisation of the genetically modified **maize MON863** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 05 June 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) allergenicity, 3) toxicity and/or 4) 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

No comments

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

Concerning plant genomic DNA flanking the insert, the applicant provided sequence information for about 1000 bp on the 5' side of the insert, and about 650 bp located on the other side. This DNA was found to be homologous to maize mitochondrial DNA. It is not known whether these sequences were inserted together with the insert during transformation, or were already present before plant transformation. In the discussion about the likelihood of the production of additional proteins, the applicant states "The likelihood of a novel protein being produced from DNA sequences encompassing the junction of the mitochondrial DNA flanking the insert in MON 863 and the maize genomic DNA to which the mitochondrial DNA is associated at either the 3' or the 5' end of the inserted DNA is also considered to be negligible." To justify this conclusion the applicant claims that lack of proper codon usage and lack of polyadenylation signal would make non functional any mRNA arising from the transcription of this sequence. These claims are difficult to follow.. Indeed AT-rich regions are not rare in prokaryotic and mitochondrial DNA, so the presence of fortuitous poly-A signals in the flanking mitochondrial DNA cannot be considered as unlikely. In addition, although codon usage can indeed be responsible for low expression level, a chimerical sequence can be expressed to moderate levels from a promoter located in plant genomic DNA; and this can lead to a significant protein accumulation. Another possible consequence of an insertion of mitochondrial DNA into plant genomic DNA is the disruption of normal plant coding sequences leading to a null mutation, or the modification of gene regulatory sequences leading to increased or decreased expression level of the gene(s). It is to evaluate such unforeseen effects that EFSA guidelines for risk assessment of GMP state that sequencing flanking DNA should extend into plant genomic DNA. However I agree that this may be a heavy task if a long stretch of mitochondrial DNA is inserted on both sides of the insert. I would rather justify the low likelihood of any safety hazard by the following arguments :

- insertion of mitochondrial DNA into plant genomic DNA is known to occur in many plant species, including maize
- the long history of safe use of a plant like maize makes unlikely that such events would lead to the production of harmful proteins.

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

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

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)

Comment 1

No comments

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

D.7.1 Comparative assessment

Comments/Questions of the expert(s)

Comment 1

I agree with the conclusion of the applicant that the minor differences in composition found between MON 863 and its control line are unlikely to be of biological significance.

Comment 2

a) Composition analysis of maize forage (Ridley et al., 2002; George et al., 2003a; George et al., 2003b).

Proximates		**Minerals**	
moisture	X	calcium	
protein	X	copper	
fat	X	iron	
ash	X	magnesium	
carbohydrates	X	manganese	
acid detergent fiber (ADF)	X	phosphorus	
neutral detergent fiber (NDF)	X	potassium	
total detergent fiber (TDF)		selenium	
starch		sodium	
		zinc	
		total nitrogen	
		chlorine	

b) Composition analysis of maize grain (Ridley et al., 2002; George et al., 2003a; George et al., 2003b).

Proximates		**Minerals**	
moisture	X	calcium	X
protein	X	copper	X
fat	X	iron	X
ash	X	magnesium	X
carbohydrates	X	manganese	X
acid detergent fiber (ADF)	X	phosphorus	X
neutral detergent fiber (NDF)	X	potassium	X
total detergent fiber (TDF)		selenium	
starch		sodium	
		zinc	X
		total nitrogen	
		chlorine	

Vitamins		Amino acids		Fatty acids		Secondary metabolites		Antinutrients	
A (β-carotene)		alanine	X	8:0 caprylic		ferulic acid	X	phytic acid	X
B1 (thiamine)	X	arginine	X	10:0 capric		furfural		raffinose	X
B2 (riboflavin)	X	asparagine		12:0 lauric		inositol	X	trypsin inhibitor	X
B3 (niacin)		aspartic acid	X	14:0 myristic		*p*-coumaric acid	X	gossypol	
B4 (choline)		cysteine	X	14:1 myristoleic				malvalic acid	
B5 (pantothenic a)		glutamic acid	X	15:0 pentadecanoic				sterculic acid	
B6 (pyridoxine)		glycine	X	15:1 pentadecenoic				dihydrosterculic acid	
B9 (folic acid)	X	histidine	X	16:0 palmitic	X				
C (ascorbic acid)		isoleucine	X	16:1 palmitoleic					
E (α-tocopherol)	X	leucine	X	17:0 margaric					
Cryptoxanthin		lysine	X	17:1 heptadecenoic					
		methionine	X	18:0 stearic	X				
		phenylalanine	X	18:1 oleic	X				
		proline	X	18:2 linoleic	X				
		serine	X	18:3 linolenic	X				
		threonine	X	20:0 arachidic	X				
		tryptophan	X	20:1 gadoleic	X				
		tyrosine	X	20:2 eicosadienoic					
		valine	X	20:3 eicosatrienoic					
				20:4 arachidonic					
				20:5 eicosapentaenoic					
				22:0 behenic	X				
				22:1 erucic					
				22:5 docosapentaenoic					
				22:6 docosahexaenoic					
				24:0 lignoceric					

There were no statistically significant differences in 240 of the 290 comparisons made between MON 863 and the non-transgenic control, MON 846, grown at the same sites.
Of the 50 comparisons found to be statistically different, 5% or approximately 15 (0.05 x 290) were expected based on chance alone.
The ranges of values for MON 863 for 90% of the statistically significant differences (45 out of 50) were found to fall within the 99% tolerance interval for the 10 commercial maize hybrids planted in 1999. The remaining five significant differences were in vitamin B2, p-coumaric acid, ferulic acid, NDF and phytic acid in grain and were observed only from one site each (MON 863 and the conventional control maize were grown at four replicated field sites). Therefore, the five significant differences in vitamin B2, p-coumaric acid, ferulic acid, fibre (neutral detergent) and phytic acid were considered to be anomalous and unlikely to be biologically meaningful.

Comment 3

A lot of references related to scientific papers were not evaluated. Three unpublished studies were evaluated, i.e. one on rats (Lemen, 2002), and 2 on mice (Naylor, 1992; Pyla (2001). The same remark is relevant for these 3 studies, i.e. based on the available values of mean and standard

deviation, it has to be concluded that the number of replications is too low to allowing statistical sound conclusions, based on the method of Berntson (1991).

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

No problems

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

OK

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

One trial with broilers was available (Taylor, 2001), but due to lacking information on standard deviations, it was not possible to evaluate the power of the statistical analysis. The feed conversion ratio was not correctly calculated, since the exact number of birds per cage were not taken into account.

D.7.5 Product specification

Comments/Questions of the expert(s)

Comment 1

No questions

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1

Comment: from a nutritional point of view, calling the protein fraction of maize “gluten” (e.g. Part I, page 88) is somewhat misleading as gluten is a composite of the proteins gliadin and glutenin . Gluten

can cause in some people gluten-sensitive enteropathy. Maize is considered acceptable for a gluten-free diet as it does not contain gluten but zein as major storage protein (Cabra et al., 2008). It is realized however that the term (corn) gluten is sometimes used for maize protein. Of course, no health problems are due to the use of the terminology in the application.

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

I agree with the conclusion of the applicant that there is no reason to believe that MON 863 would affect the intake of corn(products).

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

The disappearance of the ~3kDa peptide formed during stimulated gastric digestion is different between the MON 863 Cry3Bb1 protein (15 min) and the E. coli –produced Cry3Bb1 protein (2 min) (Part I, page 127). Does this indicate that these 2 proteins may not be equivalent? In other words: is the E. coli–produced Cry3Bb1 protein an ideal substitute for the safety evaluation of the MON 863 Cry3Bb1 protein?

Comment 2

a) Cry3Bb protein measured in MON 863 (Dudin et al., 2001).

Growth stage/ Tissue	**ng/mg Tissue Fresh Weight**		**Standard deviation**
	Mean (n)	**Range**	
Young leaf (21 days)	81 (4)	65-93	11
Forage (90 days)	39 (4)	24-45	10
Mature root (90 days)	41 (4)	25-56	13
Grain 125 days)	70 (4)	49-85	17
Silk (58 days)	10 (1)		
Pollen (60 days)	62 (13)	30-93	18

Please provide data based on dry weight.

b) NPT II protein measured in MON 863 (Dudin et al., 2001).

Growth stage/ Tissue	ng/mg Tissue Fresh Weight		Standard deviation
	Mean (n)	Range	
Young leaf (21 days)	0.98 (4)	0.74-1.4	0.27
Forage (90 days)	0.19 (4)	0.17-0.23	0.03
Mature root (90 days)	Not Analyzed		
Grain 125 days)	< 0.076 (=LOD) (4)		
Silk (58 days)	N.A.		
Pollen (60 days)	N.A.		

Please provide data based on dry weight.

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

Fast digestion of proteins in in vitro conditions does not exclude that some intact protein can reach all sites of the intestinal tract. This is a general comment valid for all proteins. In case of Cry3Bb1 protein, one of the newly expressed proteins in MON 863, it has even been found intact in feces of laying hens fed MON 863(Scheideler et al. 2008). However, based on all the arguments given by the applicant, I do not believe that this recent finding (after completion of the dossier by Monsanto in April 2007 for renewal of the authorisation) poses a hazard.

Comment 2

a) Degradation of the Cry3Bb protein in simulated gastric fluid (???).

The in vitro digestibility of MON 863 Cry3Bb1 protein and the Cry3Bb1 protein purified from E. coli has been studied using a simulated gastric fluid (SGF) mammalian digestion model. The results of this study show that the Cry3Bb1 proteins produced from MON 863 maize and from E. coli are digested to levels below the limit of detection within 15 seconds, the first analysis time point. Digestion of proteins from both sources in SGF produced a faint band corresponding to a low molecular weight peptide of 3 kDa within 15 seconds. This fragment of the Cry3Bb1 protein was digested to below the detection limit of the assay within 15 minutes in the case of the MON 863 Cry3Bb1 protein and within 2 minutes in the case of the E. coli-produced Cry3Bb1 protein.

What could be the reason for this difference?
No reference available.

b) Degradation of the Cry3Bb protein in simulated intestinal fluid (???).

No data provided.

c) Cry3Bb: Acute Oral Toxicity Study in Mice (Pyla et al., 2001).

The delivered dosages were 400, 1100, and 3200 mg MON 863 Cry3Bb1 protein per kg body weight. The delivered dosage of BSA (bovine serum albumin) protein administered to the protein control group was 2900 mg/kg body weight.
In summary, the following results were observed:

- Mortality. No mortality occurred during the study.
- Clinical observations. No test dose-related observations noted during the study.
- Body weight. No statistical differences were observed in the body weight or the body weight gain data.
- Food consumption. From day 1 to 7 of the study, a significant increase in food consumption was noted for the 400 mg/kg and the 1100 mg/kg test protein male groups compared to the vehicle control group.
- Gross necropsy data. No significant gross internal findings were observed at necropsy on study day 14.

To conclude, no adverse effects were observed which could be attributed to the oral administration of E. coli-produced Cry3Bb1 variant protein in male and female mice at doses of 400, 1100 or 3200 mg/kg of body weight. The No-Observed- Effect-Level (NOEL) of E. coli-produced Cry3Bb1 protein administered as an acute dose by gavage to mice was considered to be at least 3200 mg/kg of body weight which was the highest dose tested.

d) Degradation of the NPT II protein in simulated gastric fluid (Ream, 1993).

The metabolic fate of the protein was evaluated in simulated gastric (pepsin, pH = 1.0) fluid. Both western blot analysis and enzymatic assays confirmed that NPTII readily degrades in simulated gastric with a half live of less than 10 seconds.

e) Degradation of the NPT II protein in simulated intestinal fluid (Ream, 1993).

The metabolic fate of the protein was evaluated in intestinal (pancreatine, pH = 7.5) fluid. Both western blot analysis and enzymatic assays confirmed that NPTII readily degrades in
intestinal fluid with a halflive between 2 and 5 minutes.

f) NPT II: Acute Oral Toxicity Study in Mice (Naylor, 1992).

The safety of the NPTII protein to mammals was also assessed by mouse gavage. Mice were administered a maximum dose of approximately 5000 mg/kg of the purified protein in one day. There was no mortality, no adverse reactions and no differences attributed to treatment in body weight or

gain or food consumption in dosed mice compared to untreated mice. No abnormal changes were observed in the tissues of mice necropsied approximately eight days after dosing.

g) Cry3Bb: Amino acid sequence homology with known toxins (Hileman et al., 2001c)

Apart from expected similarities to other known crystal (Cry) proteins found in Bacillus thuringiensis and related species, no significant structural similarities were observed. The comparisons to all known proteins affirm that the MON 863 Cry3Bb1 protein sequences are homologous to Cry proteins only.

No recent update is present because the toxin database is still the same, I presume.

h) NPT II: Amino acid sequence homology with known toxins (Hileman and Astwood, 2000b).

The results of these bioinformatics analyses are consistent with the proposition that NPTII protein is not likely to pose toxicity concerns.

No recent update is present because the toxin database is still the same, I presume.

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Comment 1

No questions

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1

A whole range of naturally occurring constituents have been analysed for establishing compositional equivalence. No extra questions.

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

General comment: I think the applicants should also refer to published material expressing doubts about the wholesomeness of their GM products, even in case they do not agree.
In case of MON 863, a scientific paper appeared in March 2007 (Seralini et al., 2007) , before the completion of the dossier for renewal of the authorisation by Monsanto in April 2007, claiming that

with the present animal study data it cannot be concluded that MON 863 would be safe. After completion of the renewal dossier, this claim was refuted by a new evaluation of the data in a study financially and technically supported by Monsanto (Douli et al., 2007). The dispute is mainly on the interpretation of advanced statisticals. As I am no expert in advanced statistics, I cannot take a stand in this discussion. Starting the publication with "Report of an expert panel" may suggest that there is general agreement on the interpretation of the data. I suggest that experts in statistics look at the paper of Seralini and of Douli.

Comment 2

a) 42-day feeding study with broiler chickens (Taylor et al.,2001).

A feeding study was undertaken to compare the wholesomeness of MON 863 maize grain to six non-transgenic commercial maize varieties, in addition to the non-transgenic control, when fed to rapidly growing Ross x Ross broiler chickens.

Performance parameters were comparable (P>0.05) for all groups (broilers fed diets of MON 863 maize, non-transgenic control maize and maize from commercially available reference varieties). In addition, broilers fed diets containing MON 863 maize had similar feed efficiency to the non-transgenic control and all reference lines, and similar adjusted feed efficiency to the non-transgenic control and two of the six commercially available reference lines (RX826 and DK539). The other four reference maize line diets had slightly

decreased adjusted feed efficiencies (on average, 1.9% lower than MON 863). However, all feed efficiency and adjusted feed efficiency values fell within historical ranges for previous broiler studies and literature ranges reported for feed efficiency.

No further testing is needed.

b) 90-day rat feeding study (Lemen et al., 2002).

Male and female Sprague Dawley rat s (20/sex/group) of approximately 6 weeks of age, were fed one of the following diets for 13 weeks: 1) diets containing 11% (w/w) or 33% MON 863 maize test grain, 2) diets containing 11% or 33% non-transgenic control maize grain, or 3) diets containing 33% maize grain from six different reference varieties.

There were no test article-related deaths or adverse clinical signs observed during the study. Body weight gain and food consumption were similar in all groups throughout the study. Clinical pathology results (chemistry, haematology, coagulation and urinalyses) showed no biologically relevant differences. Organ eights and gross pathology findings were similar among test, control and reference control groups. There were no gross or microscopic lesions attributed to a dietary regimen of high-dose concentration of MON 863 maize.

No further testing is needed.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

MON 863 was developed by Monsanto Company using a transformation vector containing a modified cry3Bb1 gene (from Bacillus thuringiensis subsp. kumamotoensis), which confers protection against certain coleopteran pests (Diabrotica spp.) .

MON 863 cry3Bb1 ORF - Wild type Cry3Bb1 (GenBank Accession No M89794) was modified to enhance its insecticidal activity against the coleopteran pest, corn rootworm. Cry3Bb1 protein has also been referred to as "CryIIIB2", "Cry3B2", or "CryIIIC". However, according to standardized nomenclature published by Crickmore (Crickmore et al., 1998), the appropriate designation for this protein is Cry3Bb1. The wild type of this protein is present in the commercial product, Raven® Oil Flowable Bioinsecticide, which has been sold in the U.S. since 1995 for control of coleopteran pests. Compared to the wild type protein sequence, MON 863 Cry3Bb1 protein differs by seven amino acids (Astwood et al., 2001) and shares almost 99% homology. Cry3Bb1 also shares approximately 67% amino acid sequence homology with another Cry3 protein, Cry3Aa4 (GenBank Accession No M30503), which has been commercially used in the U.S., E.U. and other countries for control of Colorado potato beetle, a major pest of potatoes (Perlak et al., 1993).

Maize has a history of safe use for human food and animal feed. The OECD described in its consensus document (OECD, 2002) the antinutrients present in maize (phytic acid; 2,4-Dihydroxy-7-methoxy-2H-1,4- benzoxazin-3(4H)-one; raffinose and low levels of trypsin and chymotrypsin inhibitors) but none of them are considered nutritionally significant for human health (White and Pollak, 1995). Concerning allergenicity, maize is not a common allergenic food (OECD, 2002) and very few cases of allergenic reactions to the consumption of maize products have been reported. In conclusion, the toxic and allergenic risk posed from consumption of maize and derived products is likely to be very low.

PCR and DNA sequencing of the products were performed on genomic DNA to confirm the MON 863 insert. The results of these PCR reactions are shown in Figure 15. The negative controls of MON 846, distilled water, and an unrelated transgenic maize line did not yield a PCR product when MON 863 genomic DNA yielded the correct size products of 312 bp for MON 863 sequence analysis. This demonstrates the specificity of the primer pairs to MON 863. The sequences of these PCR products were compared to sequence data previously obtained for the MON 863 insert and were confirmed. Therefore, this PCR analysis verified the nt MON 863. This sequence analysis determined that the insert begins at base 156 and ends at base 4830 in plasmid PV-ZMIR13 (Cavato and Lirette, 2001a) (or begins at base 7 and ends at base 4681 when referred to transformation fragment PV-ZMIR13L). No relevant chimearic ORF encompass the insert/plant DNA junctions and no biologically relevant homologies with sequences of allergens and/or toxins in the public databases have been identified.

The Cry3Bb1 and NPTII proteins present in MON 863 maize were studied in a series of experiments including an analysis of potential homology to known toxins and allergens and an estimation of dietary exposure (see D.7.8.). These studies demonstrated the safety of these proteins to humans and

animals, as previously reported in the applications under Regulation (EC) N° 258/97 and Directive 2001/18/EC for MON 863 maize.

This assessment of the allergenic potential of the Cry3Bb1 and NPTII proteins compares the biochemical characteristics of these proteins to characteristics of known allergens. A protein is not likely to be an allergen if:

- The protein is from a non-allergenic source;
- The protein does not share structural similarities to known allergens based on the amino acid sequence;
- The protein is rapidly digested in simulated gastric fluid;
- The protein represents only a very small portion of the total protein in the grain.

In the following sections, these four characteristics are discussed in detail for the Cry3Bb1 and NPTII proteins produced in MON 863. General information on the methods used to assess the structural similarity to known allergens and stability in simulated digestive fluids is provided below.

Rational for studying structural similarity to known allergens In 2003, the Codex Alimentarius Commission published guidelines for the evaluation of the potential allergenicity of novel proteins (Codex, 2003). The guideline is based on the comparison of amino acid sequences between introduced proteins and known protein allergens. The potential allergenic cross-reactivity may exist if the introduced protein is found to have at least 35% amino acid identity with a known allergen over any segment of at least 80 amino acids. The Codex guideline also recommended that a sliding window search with a scientifically justified peptide size, such as eight amino acids, could be used to identify immunologically relevant peptides in otherwise unrelated proteins.

Bioinformatic analyses were performed on Cry3Bb1 and NPTII proteins expressed in MON 863 to assess potential similarity to allergens and identify immunologically relevant peptides. The analyses revealed no significant matches to known allergens. Rational for studying stability in simulated digestive fluids A factor that increases the likelihood of allergic oral sensitization to proteins is the stability of the proteins to gastrointestinal digestion. Protein allergens tend to be stable to the peptic and acidic conditions of the digestive system if they are to reach and pass through the intestinal mucosa to elicit an allergenic response (Astwood et al., 1996; Metcalfe et al., 1996). Proteins that are rapidly digestible are highly correlated with a significantly decreased likelihood to cause sensitization or allergic reaction when consumed.

One aspect of this assessment includes analysis of the digestibility of the protein in a simulated gastric fluid (SGF) assay containing pepsin (Part I Technical dossier 125 Regulation (EC) No 1829/2003 MON 863 Monsanto Company). A relationship between digestibility in SGF and the likelihood of being an allergen has been previously reported with a group of proteins consisting of both allergens and non-allergens (Astwood et al., 1996). Recently, the SGF assay protocol was standardized by the International Life Science Institute (ILSI) based on results obtained from an international, multi-laboratory ring study (Thomas et al., 2004). This test showed that the results of in vitro pepsin digestion assays are reproducible when standard protocols were followed. Using these protocols, the pepsin digestion assay was used to assess the susceptibility of the Cry3Bb1 and NPTII proteins to pepsin digestion in vitro.

In addition to SGF, simulated intestinal fluid (SIF) is also used for in vitro studies to assess the digestibility of food components (Okunuki et al., 2002; Yagami et al., 2000). SIF is an in vitro digestion model where proteins undergo digestion at neutral pH by a mixture of enzymes known as pancreatin. The relationship between protein allergenicity and protein stability in the in vitro SIF study is limited, because the protein has not been first exposed to the acidic, denaturing conditions of the stomach, as would be the case in vivo (FAO/WHO, 2001). In vitro susceptibility of Cry3Bb1 and NPTII proteins to

pancreatin was assessed for digestibility in SIF according to methods described in the United States Pharmacopeia (Pharmacopoeia, 1995).

Assessment of the potential for allergenicity of the Cry3Bb1 protein

a) Source

Bacillus thuringiensis is a spore-forming, gram-positive bacterium that is found naturally in soil. B.t. strains have been used commercially in the U.S.A. since 1958 to produce microbial-derived products with insecticidal activity (US EPA, 1988). There are no known reports of allergies to B.t. species or to the proteins produced by these species.

b) Bioinformatics analyses of sequence similarity to allergens

Allergenic potential was assessed by comparing the amino acid sequence similarities of the MON 863 Cry3Bb1 protein to protein sequences within an allergen database, including known and putative allergens, using bioinformatics techniques (Hileman et al., 2001c).

The retrieval strategy employed in this study allowed the assembly of a comprehensive allergen and gliadin database. The FASTA sequence alignment tool was used to compare the MON 863 Cry3Bb1 protein sequences to this database. Results of the FASTA sequence alignments demonstrated the lack of structurally relevant similarity between the MON 863 Cry3Bb1 protein and any known allergen or gliadin. Additionally, an algorithm was developed to determine if the MON 863 Cry3Bb1 protein sequences shared a match of eight or more linearly contiguous amino acids to any sequence within the allergen and gliadin database. Results from the epitope match algorithm (IDENTITYSEARCH) demonstrated the lack of potentially immunologically relevant sequences in MON 863 Cry3Bb1 protein sequence.

The results of these bioinformatics analyses are consistent with the proposition that MON 863 Cry3Bb1 protein is not likely to pose human allergy concerns.

Updated bioinformatic analysis of the Cry3Bb1 amino acid sequence confirmed that this protein has no biologically revelant similarities to known allergens (McClain and Silvanovich, 2007). This information is provided separately in Section 3 of the supplement accompanying this application:

c) Digestibility in simulated digestive fluid

The in vitro digestibility of MON 863 Cry3Bb1 protein and the Cry3Bb1 protein purified from E. coli has been studied using a simulated gastric fluid (SGF) mammalian digestion model. A correlation between digestibility in SGF and food safety has been previously validated using this model (Astwood et al., 1996). In SGF, proteins which have been safely consumed were observed to be rapidly degraded and known food allergens were observed to be relatively stable to proteolysis by pepsin. Although the SGF model demonstrates the digestibility of a protein by mammalian digestive enzymes, it is not intended to predict the half-life of a protein in vivo. However, specific parameters in the SGF model are representative of human digestion and are widely used in nutrition studies. Proteins which are nutritionally desirable tend to be relatively digestible and will have greater bioavailability of amino acids than stable proteins. In addition, proteins that are highly digestible would be expected to have less opportunity to exert adverse health effects when consumed. Simulated gastric and intestinal models provide supportive data which can be used in combination with direct toxicity testing (i.e., acute oral toxicity in rodents) (Hammond and Fuchs, 1998) or in combination with additional parameters used to assess allergenic potential (Metcalfe et al., 1996).

The results of this study show that the Cry3Bb1 proteins produced from MON 863 maize and from E. coli are digested to levels below the limit of detection within 15 seconds, the first analysis time point. Digestion of proteins from both sources in SGF produced a faint band corresponding to a low

molecular weight peptide of 3 kDa within 15 seconds. This fragment of the Cry3Bb1 protein was digested to below the detection limit of the assay within 15 minutes in the case of the MON 863 Cry3Bb1 protein and within 2 minutes in the case of the E. coli-produced Cry3Bb1 protein.
This ~3 kDa transitory peptide of Cry3Bb1 is not expected to be capable of eliciting an IgE-mediated allergic reaction. The minimum structural requirement for eliciting an effective clinical response through mast cell degranulation is the presence of two high affinity IgE binding epitopes separated by a relatively rigid spacer molecule of 80 angstroms (Holowka and Baird, 1996). The hypothetical minimum requirement for a peptide to elicit an allergic response would be a six (to 15) amino acid linear epitope on each end separated by 16 amino acids based on the assumption that each residue has a diameter of at least 5 angstroms (size for Ala), for a total of 28 amino acids. Even though the longer of the two potential Cry3Bb1 peptides approaches this length (27 aa), the probability of having two high affinity epitopes at the extreme ends that were not identified by the sequence search, yet could trigger a response, is remote. Cry3Bb1 proteins are highly digestible and would be expected to have less opportunity to exert adverse health effects when consumed relative to known toxins and allergens. Rapid digestion of the intact Cry3Bb1 protein in SGF is a characteristic shared among proteins with a history of safe consumption, but conversely, the major food allergens that have been tested in the SGF model are stable to digestion (del Val et al., 1999); (Astwood et al., 1996); (Taylor and Lehrer, 1996); (Deshpande and Neilsen, 1987). Comparison of these digestion patterns with the in vitro digestion model cited (Astwood et al., 1996) indicates that the Cry3Bb1 proteins fall within the digestion patterns observed for other proteins. The MON 863 Cry3Bb1 protein is highly digestible in the SGF and therefore will already be digested when reaching the intestine. Nevertheless, the in vitro digestibility of E. coliproduced MON 863 Cry3Bb1 in simulated intestinal fluid (SIF) has been investigated although it not relevant to the safety assessment of this protein. As expected, the Cry3Bb1 protein was observed to rapidly degrade (within 1 minute) from a size of approximately 74kDa to smaller fragments with approximate molecular weights of 68 and 57 kDa. These molecular weights are very similar to those observed previously for Cry3Bb1 variants isolated from B.t., which correspond to N-terminally truncated forms of the full-length proteins (Hileman et al., 2001b). Continued exposure to SIF (= 5 minutes) resulted in the formation of a single stable fragment at a molecular weight of approximately 57 kDa. The molecular weight of this polypeptide is consistent with the expected size of the tryptic core for Cry3 proteins. To conclude, these studies suggest that the Cry3Bb1 protein will be readily digestible in the mammalian digestive tract. The results, in combination with the lack of sequence similarity to allergens, the lack of reported allergy to microbial pesticides that contain MON 863 Cry3Bb1 protein, and the low levels of the MON 863 Cry3Bb1 protein in maize relative to food allergens, support the conclusion that there is no increased risk of food allergy associated with exposure to the MON 863 Cry3Bb1 protein.

Assessment of the potential for allergenicity of the NPTII protein

a) Source

The NPTII protein is ubiquitous in the environment and found in microbes present in food and within the human digestive system. This protein has also been widely used as a selectable marker for animal cell transformation and for human gene therapy experiments (Brenner et al., 1993; Culver et al., 1991). There are no known reports of allergies to NPTII protein.

b) Bioinformatics analyses of sequence similarity to allergens

A study was undertaken to compare the amino acid sequence similarities of the NPTII protein to protein sequences within an allergen database using bioinformatics techniques (Hileman and Astwood, 2000a). The retrieval strategy employed in this study allowed the assembly of a comprehensive allergen and gliadin database. The FASTA sequence alignment tool was used to

compare the NPTII protein sequences to this database. Results of the FASTA sequence alignments demonstrated the lack of structurally relevant similarity between the Cry3Bb1 proteins and any known allergen or gliadin. Additionally, an algorithm was developed to determine if the NPTII protein sequence shared a match of eight or more linearly contiguous amino acids to any sequence within the allergen and gliadin database. Results from the epitope match algorithm (IDENTITYSEARCH) demonstrated the lack of any potentially immunologically relevant NPTII protein sequence. The results of these bioinformatics analysis are consistent with the proposition that NPTII protein is not likely to pose human allergy concerns.
Updated bioinformatic analysis of the NPTII amino acid sequence confirmed that this protein has no biologically relevant similarities to known allergens (McClain and Silvanovich, 2007). This information is provided separately in Section 3 of the supplement accompanying this application: "3.5. Updated information on allergenicity and toxicology".

c) Digestibility in simulated digestive fluid
The metabolic fate of the protein was evaluated in simulated gastric (pepsin, pH = 1.0) and intestinal (pancreatine, pH = 7.5) fluids (Ream, 1993). Both western blot analysis and enzymatic assays confirmed that NPTII readily degrades in simulated gastric and intestinal fluids with respective halflives of less than 10 seconds and between 2 and 5 minutes. Additional information on putative peptides encoded by all reading frames at the 5' and 3' junctions of the MON 863 insertion event and plant genomic DNA Bioinformatics analyses were performed to assess putative peptides encoded by all reading frames at the 5' and 3' junctions of the MON 863 insertion event and plant genomic DNA for their potential similarity towards known allergens, toxins or other pharmacologically active proteins relevant to human and animal health (Hileman et al., 2002). DNA sequences flanking the insertion event were translated from stop-to-stop codon in all six reading frames for the 5' end (5a-f), and in all six reading frames for the 3' end (3a-f). Putative peptides spanning these junctions were compared to allergen (AD3_1), toxin (TOXIN5) and public domain (ALLPEPTIDES) sequence databases using bioinformatic tools.
The FASTA sequence alignment tool was used to assess structural similarity shared between each putative polypeptide with sequences in the databases. The extent of similarity was evaluated by visual inspection of the alignment, the calculated percent identity and the E score value. The E score reflects the degree of similarity and the value depends on the overall length of joined (gapped) local sequence alignments, the quality (percent identity, similarity) of the overlap and the size of the database. A larger E score value indicates a lower degree of similarity between the query sequence and the sequence from the database. In addition to structural similarity, each putative polypeptide was screened for short peptide matches using a pair-wise comparison algorithm. In these analyses, eight linearly contiguous and identical amino acids were defined as immunologically relevant, where eight represents the typical minimum sequence length likely to represent an immunological epitope (IgE). No biologically relevant structural similarities to allergens were observed for any of the putative peptides. Further, no short (eight amino acid) peptide matches were shared between any of the putative peptides and proteins in the allergen database. No biologically relevant structural similarities to toxins were observed for any of the putative peptides. Comparisons to all publicly available sequences revealed that reading frame 5b, corresponding to the authentic reading frame of the nptII expression cassette, contained a sequence homologous to the cauliflower mosaic virus 35S promoter, as expected. No other biologically relevant structural similarity was observed. These data demonstrate the lack of both structurally and immunologically relevant similarities towards allergens for all of the putative peptides analyzed. These data also demonstrate the lack of structurally relevant similarities towards toxins or other pharmacologically active proteins for all of the putative peptides analyzed.

7.9.2 Assessment of allergenicity of the whole GM plant or crop

Maize is not considered to be a common allergenic food. Food allergies to maize are of low frequency and mainly occur in populations of specific geographic areas. Rare cases of occupational allergy to corn dust have been reported. There is no reason to expect that the use of MON 863 maize will significantly increase the intake and exposure to maize. Therefore a possible over expression of any endogenous protein, which is not known to be allergenic, would be unlikely to alter the overall allergenicity of the whole plant or the allergy risk for consumers.

Remarks

A recent paper (Untersmayr and Jensen-Jarolim, 2008) relativates the role of gastric fluid digestibility in assessing the allergenic potential of food. Furthermore, the increased (ab)use of stomach acid reducers (anti H2- antihistamines, proton pump inhibitors) has to be taken into account. Follow up studies evaluating the emergence of allergy are mandatory.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1

No questions

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)

NOT APPLICABE

D.9. POTENTIAL CHANGES IN THE INTERACTIONS BETWEEN THE GM PLANT WITH THE BIOTIC ENVIRONMENT RESULTING FROM THE GENETIC MODIFICATION

NOT APPLICABE

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

NOT APPLICABE

D.11. ENVIRONMENTAL MONITORING PLAN

NOT APPLICABE

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