Bioveiligheidsraad
Conseil de Biosécurité

16/12/2011

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2012_0034

Title: Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/RX-MON531 from Monsanto under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/RX-MON531 was submitted by Monsanto on 29 June 2007 for renewal of the authorisation of (1) foods produced from genetically modified cotton MON 531 (food additives) and (2) feed produced from genetically modified cotton MON 531 (feed materials and feed additives) within the framework of Regulation (EC) No. 1829/2003[1]. On 30 June 2011, the European Commission acknowledged the applicant's request to expand the scope also to foods produced from cotton MON 531 (cottonseed oil).
Cotton MON531 expresses the Cry1Ac insecticidal protein conferring resistance to specific lepidopteran cotton pests, as well as genes coding for neomycin phosphotransferase type II (NPTII) and 3′(9)-O-nucleotidyltransferase (AAD, not expressed in MON 531), which were used as antibiotic resistance marker genes during product development.

The application was officially acknowledged by EFSA on 11 June 2008. On the same date EFSA started the formal three-month consultation period of the Member States, in accordance with Articles 6.4 and 18.4 of Regulation (EC) No. 1829/2003 (consultation of national Competent Authorities within the meaning of Directive 2001/18/EC designated by each Member State in the case of genetically modified organisms being part of the products).

Within the framework of this consultation, the Belgian Biosafety Advisory Council (BAC), under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier, chosen from the common list of experts drawn up by the BAC and the Biosafety and Biotechnology Unit (SBB). Five 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 placed on the EFSAnet on 11 September 2008.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 7 September 2011 (EFSA Journal, 2011;9(9):2373)[2], and published on 16 September 2011 together with the responses from the EFSA GMO Panel to comments submitted by the experts during the three-month consultation period.

On 21 September 2011 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

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed (OJ L 268, 18.10.2003, p.1)
[2] See <http://www.efsa.europa.eu/en/efsajournal/pub/2373.htm>

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

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

Scientific evaluation

1. Environmental risk assessment

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

2. Molecular characterisation

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

3. Assessment of food/feed safety and nutritional value

3.1. Assessment of compositional analysis

The compositional analysis performed by the applicant has not included the analysis of Vitamin E in cottonseed, as recommended by the OECD consensus document on compositional considerations for new varieties of cotton[4].

3.2. Assessment of toxicity

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

3.3. Assessment of allergenicity

As the allergenicity of the whole GM cotton has not been assessed, it is recommended to take up monitoring of allergenicity as part of the general surveillance.

3.4. Nutritional value

The Biosafety Advisory Council is of the opinion that the information provided is sufficient and shows the nutritional equivalence of the GM cotton with its non-GM counterpart and conventional cotton varieties.

4. Monitoring

With regard to monitoring, the Biosafety Advisory Council is of the opinion that the information provided is sufficient.

[3] As the application doesn't imply a cultivation of the GM crop in the EU, a full environmental assessment is not required in EFSA procedure and was not achieved

[4] Consensus Document on Compositional Considerations for New Varieties of Cotton (Gossypium hirsutum and Gossypium barbadense): Key Food and Feed Nutrients and Anti-Nutrients. ENV/JM/MONO(2004)16 - Revised version of December 2009

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 is of the opinion that the applicant did not follow the OECD recommendation on the comparative compositional analysis regarding the content of Vitamin E in the seeds and did not argue why not.

However, in case the applicant can prove that the analysis of alfa tocoferol in the oil (which is the analysis the applicant performed) is a good proxy for the analysis required by the OECD, the Biosafety Advisory Council can accept the presented data as a good basis to give a positive advice regarding the biosafety of the event.
Based on the currently available information, the Biosafety Advisory Council does not give an advice on the health safety of the GMO.

The Biosafety Advisory Council did not identify any risk that the import and processing of this GM cotton could pose to the environment.

In addition, the Biosafety Advisory Council recommends following up any unanticipated allergenicity aspects of the GM cotton in monitoring systems.

p.o. Dr. Ph. Herman

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

Annex I: Full comments of experts in charge of evaluating application EFSA/GMO/RX-MON531 and comments submitted on the EFSAnet (ref. BAC_2008_806)

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Secretariaat
Secrétariat

N./réf. : WIV-ISP/BAC_2008_806
Email. : bac@sbb.ihe.be

11-09-2008

Compilation of comments of experts in charge of evaluating the application EFSA/GMO/RX-MON531
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. Philippe Baret
Experts: Pascal Cadot (Consultant), Armand Christophe (UGent), Rony Geers (KUL), Peter Smet (Consultant), Wim Stevens (UIA)
Domains of expertise of experts involved: human nutrition, animal nutrition, biochemistry of food/feed, additives for food/feed, toxicology, immunology, alimentary allergology
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

INTRODUCTION

Dossier **EFSA/GMO/RX-MON531** concerns an application of the company **Monsanto** for the renewal of marketing authorisation of the genetically modified **cotton MON531** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 11 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)

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

Comments/Questions of the expert(s)

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

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)

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

Comments/Questions of the expert(s)

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

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

D.7.1 Comparative assessment

Comments/Questions of the expert(s)

Comment 1

a) Composition analysis of cottonseed (Oberdoerfer, 2006b, Rattemeyer-Matschurat, 2006b).

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

Vitamins		Amino acids		Fatty acids		Secondary metabolites		Antinutrients	
A (β-carotene)		alanine	X	8:0 caprylic		ferulic acid		phytic acid	
B1 (thiamine)		arginine	X	10:0 capric		furfural		raffinose	
B2 (riboflavin)		asparagine		12:0 lauric		inositol		trypsin inhibitor	
B3 (niacin)		aspartic acid	X	14:0 myristic	X	*p*-coumaric acid		gossypol	X
B4 (choline)		cysteine	X	14:1 myristoleic				malvalic acid	X
B5 (pantothenic a)		glutamic acid	X	15:0 pentadecanoic	X			sterculic acid	X
B6 (pyridoxine)		glycine	X	15:1 pentadecenoic				dihydrosterculic acid	X
B9 (folic acid)		histidine	X	16:0 palmitic	X				
C (ascorbic acid)		isoleucine	X	16:1 palmitoleic	X				
E (α-tocopherol)		leucine	X	17:0 margaric	X				
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					
		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	X				

The composition of cottonseed (proximate analysis, lipid content, fatty acid composition) of MON531 is comparable to the Coker 312 control variety and to published ranges for other cotton varieties.
Levels of the three toxicants (gossypol, cyclopropenoid fatty acids and aflatoxin) for MON531 are comparable to Coker 312.
Cottonseed from MON531 processed comparably to the Coker 312 control, with comparable reductions in the levels of gossypol in the processed meal for both lines. As expected, there was no gossypol in refined cottonseed oil.
The levels of the important fatty acids and protein components as well as the toxicants, gossypol and cyclopropenoid fatty acids, in the oil are also comparable.

The quality of the processed cottonseed products from MON531 was shown to be equivalent to the Coker 312 control line. The Cry1Ac protein was not detected in raw cotton fiber, cleaned cotton fiber or cleaned linters. Refined oil from MON531 and Coker 312 showed no detectable levels of protein.

Additional comment from SBB

In previous cotton dossiers, the following comments were also made:

- Phytic acid has not been determined. Why not?
- Vitamin E concentrations were not measured. However, it is on the list of essential nutrients in cotton of the OECD, and therefore should be measured.

Comment 2

- Table 10: The reported calories for cottonseed in Table 10, Part I, page 104 (422 kcal/100g) are probably calculated using an inappropriate formula. As carbohydrate values were determined by calculation (Annex 3.4.c, page 14) they include fiber. Using an energy factor of 4kcal/g for "carbohydrate", as was probably done, may not be appropriate. Idem for annex 3.4, page 18, Table 1. Such calculated energy values may be of little nutritional significance. Indeed, net energy of whole cottonseed was reported to be around 200 kcal/100g. (Bertrand JA, Sudduth TQ, Condon A, Jenkins TC, Calhoun MC, 2005. Nutrient content of whole cottonseed. J Dairy Sci 88:1470-1477.).

- There seems to be a contradiction between the values in Table 10 (Part I, page104) (fat MON 531 = 20.8%; moisture 13.5%) and in Table 12 (lipid MON531 expressed on lyophylized weight = 39.97%??). Is there an explanation?

- Minor remarks:

1) Two different positional isomers of octadecenoic acid (18:1n-7 and 18:1n-9) have been found in cottonseed oil. (Radcliffe JD, Czajka-Narins DM, Imrhan V, 2004. Fatty acid composition of serum, adipose tissue, and liver in rats fed diets containing corn oil or cottonseed oil. Plant Foods Hum Nutr 59:73-77.). In Tables 13 and 21 "oleic acid" is used for 18:1 which is name of the 18:1n-9 isomer. Is this a misnamer or is the other isomer not found? Of course this is a minor remark which has no bearing on the nutritional properties.
2) The saturated fatty acid with 17 carbon atoms, shorthand notation 17:0, is not haptadecanoic acid as mentioned on page 15, lines 4 and 6 of annex 3.4 , but heptadecanoic acid.
3) It does not make sense to compare the fatty acid composition of an oil with that of its phospholipids fraction as is done for arachidic acid (reference with superscript 7 in Table 3 from Annex 3.4, page 25). In contrast with what is claimed in the annex, arachidic acid is not expressed in the cited publication as % phospholipids in the oil but as % of the phospholipd fatty acids in the oil (which make up about % of the phospholipids).

Comment 3

Papers already published in scientific journals were not evaluated. One study with mice (Sammons, 1994) could not be evaluated since no information on the variability in the results was provided. Another study with rats (Naylor, 1992) seems to meet the requirements with respect to the number of replications, at least when combining both sexes in one analysis.

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

Is OK

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

- Gossypol level of MON531 seed is in the normal range but the biological active gossypol is free gossypol (Mena et al., 2004) and anti-fertility effects in male mammals are dependent on the enantiomer type. Oligospermia in men has been observed at an intake of 20 mg of gossypol per day (Lopez et al., 2005). No results were given on the free fraction nor on the enantiomeric composition of gossypol in whole seed. The latter may not be relevant to humans as they are not expected to consume whole cottonseed. (Free gossypol was determined in toasted meal and in refined oil).

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

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

No questions. I agree with the conclusion of the applicant that the use of MON531 for the production of food and feed is not expected to be any different from that of conventional cotton.

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

No questions. I agree with the conclusion of the applicant (Part I, page122).

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

a) Cry1Ac protein measured in MON531 (Specific info; 3.04_Protein_&_Compo.pdf).

Growth stage/ Tissue	**ng/mg Tissue Dry Weight**		**Standard deviation**
	Mean	**Range**	
Young leaves (3-6 week plantlets)	12	< LOQ – 24	8.7
Seed	1.8	1.7 – 1.9	0.086

b) NPT II protein measured in MON531 (Specific info; 3.04_Protein_&_Compo.pdf).

Growth stage/ Tissue	**ng/mg Tissue Dry Weight**		**Standard deviation**
	Mean	**Range**	
Young leaves (3-6 week plantlets)	41	< LOQ – 41	*
Seed	5.7	5.1 – 6.5	0.53

* NPTII results were based on one site because the values from the other sites were less than the assay limits of quantitation (LOQ).

c) AAD protein measured in MON531 (Specific info; 3.04_Protein_&_Compo.pdf).

The aad sequence is controlled by its own bacterial promoter; therefore, the protein was not expected to be expressed in cotton tissues from MON531. The absence of AAD protein expression was subsequently confirmed using ELISA analyses.

Comment 2

No questions

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

Safety assessment of newly expressed proteins.
The test proteins were produced in E. coli, purified, characterized relative to the plant produced protein, and shown to be chemically and functionally equivalent to the plant produced proteins.

a) Degradation of the Cry1Ac protein in simulated gastric fluid **(???)**.

After approximately 30 sec incubation in gastric fluid, no intact Cry1Ac protein was detected by western blot analysis and less than 50% of the tryptic core (formed by the degradation of intact Cry1Ac protein by pepsin) was detected. Greater than 90% of the Cry1Ac protein bioactivity against tobacco budworm dissipated at all three concentrations tested after approximately five min of incubation in gastric fluid.

There seems to be something wrong with the references. The technical dossier mentions the reference Ream, 1994, whereas this document is entitled "Aerobic soil degradation…"

b) Degradation of the Cry1Ac protein in simulated intestinal fluid **(???)**.

In simulated intestinal fluid, the Cry1Ac protein readily degraded to its tryptic fragment. No Cry1Ac protein was detected by western blot analysis after 30 min incubation. The tryptic fragment, formed from added Cry1Ac protein, did not degrade significantly after approximately 21 h incubation in intestinal fluid as detected by western blot analysis. This result is consistent with the bioassay, which also indicated no significant loss of activity against tobacco budworm after approximately 21 h incubation in intestinal fluid.

There seems to be something wrong with the references. The technical dossier mentions the reference Ream, 1994, whereas this document is entitled "Aerobic soil degradation…"

c) Cry1Ac: Acute Oral Toxicity Study in Mice **(???).**

The doses of Cry1Ac protein administered to mice were 0, 500, 1000, and 4200 mg/kg.
Another group of mice was gavaged with 6340 mg/kg bovine serum albumin (BSA) and served as a protein control group. Since the Cry1Ac test material was 68% pure, it was considered necessary to dose mice with a higher dose of BSA to make the total mass of material given to mice equivalent for the BSA (protein) control and test group.

There were no treatment related adverse findings in any of the groups of mice administered Cry1Ac protein or control materials by oral gavage at dosages up to 4200 mg/kg.

There were no statistically significant differences in body weight, cumulative body weight, or food consumption between the vehicle or protein control groups and Cry1Ac protein treated groups.

No reference is available.

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

Both western blot analysis and enzymatic activity assays confirmed that NPTII readily degrades in simulated gastric with a half-live of less than ten seconds.

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

Both western blot analysis and enzymatic activity assays confirmed that NPTII readily degrades in intestinal fluids with a half-live between two and five minutes.

f) NPT II: Acute Oral Toxicity Study in Mice (Fuchs et al., 1993c).

Mice were administered a maximum dose of approximately 5000 mg/kg of the purified NPTII protein in one day. There were no mortality, no adverse reactions, and no differences attributed to treatment in body weight 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) Cry1Ac: Amino acid sequence homology with known toxins (Silvanovich et al., 2002)

File was damaged and the software could not repair it. Received a copy but the problem remained.

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

The safety assessment of a protein expressed in genetically modified crops includes structural comparisons of the amino acid sequence of the introduced protein with proteins associated with toxicity or other adverse health effects. Specifically, a biologically relevant sequence similarity to a known toxin (i.e., a sequence apparently derived from a common ancestor gene) may indicate that additional toxicological assessments be done.
A database of 4677 protein sequences associated with toxicity was assembled from publicly available genetic databases (GenBank, EMBL, PIR and SwissProt). The amino acid sequence of the NPT II protein was compared with this toxin sequence database using the FASTA sequence alignment tool. In addition, the amino acid sequence of the NPT II protein was compared with all protein sequences in publicly available genetic databases to screen for structural similarity to pharmacologically active proteins. NPT II shared sequence similarities to homologous aminoglycoside modifying enzymes, as expected.
No other significant structural homology was observed.
The results of these bioinformatics analyses indicate that the NPT II protein is not similar to any toxin relevant to animal or human health.

Comment 2

No questions

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

No questions

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

a) 42-day feeding study with broiler chickens ().

Not performed. No further testing is needed.

b) 90-day rat feeding study ().

Not performed. No further testing is needed.

c) One-month feeding study in rats (Naylor, 1992)

Unprocessed, Transgenic (Line 81) and Non-Transgenic (Coker 312) Cottonseed Meal was administered in feed to Sprague-Dawley rats at target levels of 0, 5 and 10% by weight of the total diet (0, 50,000 and 100,000 parts per million, respectively) for approximately one month. (Line 81 is the genetically-transformed line of Coker 312 Cotton.) Clinical observations, body weights and food consumption were performed weekly. All animals were necropsied at study termination and their kidneys, liver and testes were weighed. Selected tissues were retained, but were not examined microscopically.
Overall study averages for test material consumption, based on the target concentrations, were approximately 3519 and 6587 in males, and 3731 and 7240 in females [mg Non-Transgenic Cottonseed Meal (Coker 312)/kilogram body weight/day], and 3523 and 6676 in males, and 3735 and 7569 in females [mg Transgenic Cottonseed Meal (Line 8l)/kilogram body weight/day]. There were no treatment-related effects on survival, clinical observations or gross pathology.

Substitution of cottonseed meal apparently rendered the resulting diet less palatable to the rats, particularly at the 100,000 ppm level. Food consumption of both Transgenic and Non-Transgenic meal diets was significantly reduced in both sexes for the first two weeks at the 100,000 ppm level. Consumption of the Transgenic meal diet at the 50,000 ppm level was also reduced in females for the first week of testing. When compared to each other, however, there was no significant difference in food consumption for rats of either sex fed Transgenic or Non-Transgenic diets. Cumulative body weight gain was also reduced compared to the untreated controls in most groups given either cottonseed meal diet at either dosage; however, animals given Transgenic meal diet gained approximately the same amount of weight as those given the Non-Transgenic meal diet.
The few absolute organ weight changes, smaller livers in males at the highest dietary levels and smaller kidneys in females at the highest dietary level of either the Non-Transgenic or Transgenic diet, were attributed to the decreased growth of these animals. The changes in relative organ weights, increases of testes and kidneys in males and liver in females at the 100,000 ppm dietary level as compared to the untreated control organ weights, were also considered due to reduced body growth. There were no significant, changes in organ weights, either absolute or relative, when the comparison between animals given Transgenic and Non-Transgenic meal diets was analyzed.
Based on the above results, there did not appear to be any biologically significant differences in the measured parameters (growth, food consumption, organ weights and gross pathologic examination), between animals fed Transgenic or Non-Transgenic Cottonseed meal at either 50,000 or 100,000 ppm in the diet.

This test is not mentioned in the technical dossier.
Is line 81 the same as MON531?
Additional comment from the SBB:
The abovementioned reference (technical report) is indeed provided in the dossier (CI) but not referred to in the technical dossier. We did not found any information regarding the link between line 81 and line 531 (neither in this dossier nor in the first dossier submitted in 1997 by Monsanto in the framework of Regulation 258/97). To our understanding, line 81 is most probably a transformation event different from MON531. But this could be checked with the applicant.

Comment 2

No questions

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

Synthesis of provided data:

MON531 was developed by Monsanto Company through genetic modification of conventional cotton and has been commercialized as Bollgard® cotton.

MON531 contains the genetic material necessary to express the Cry1Ac and NPTII proteins. The Cry1Ac protein confers protection from specific lepidopteran insect pests and NPTII allows for

selection on a kanamycin containing media during the transformation process. Additionally, MON531 contains the aad coding sequence, however, the sequence is under the control of a bacterial promoter, and therefore, the aad coding sequence-product is not produced. MON531 was produced using Agrobacterium tumefaciens mediated transfer of the cry1Ac coding sequence into the genome of a conventional cotton variety, Coker 312, using a binary plasmid vector. The cry1Ac coding sequence was derived from the common soil bacterium Bacillus thuringiensis subsp. kurstaki HD-73 (B.t.k.), and encodes the Cry1Ac protein that confers resistance to lepidopteran pests. The nptII coding sequence, which encodes a selectable marker enzyme, neomycin phosphotransferase II (NPTII), was also present on the plasmid to facilitate selection of insect-protected plants on a kanamycin containing media. The nptII coding sequence was isolated from the prokaryotic transposon Tn5. The NPTII protein served no other purpose and has no pesticidal properties. The plasmid also contained the antibiotic resistance coding sequence 3′(9)-O-aminoglycoside adenylyltransferase (aad), isolated from transposon Tn7. The aad sequence confers resistance to the antibiotics spectinomycin and streptomycin, and facilitated the selection of bacteria containing the plasmid in the initial steps of transforming the cotton tissue. The aad coding sequence is under the control of a bacterial promoter and the encoded protein is not detected in MON531 plant tissue.

Cottonseed is processed into four major products: oil, meal, hulls, and linters. On average, refined cottonseed oil accounts for approximately 40-50% of the total value of all four products (NCPA, 2006b). Cottonseed oil and to a lesser extent processed linters are routinely used in human food and have a long history of safe use (FDA/CFSAN, 2002). Whole cottonseed, cottonseed meal, and hulls are used in animal feed (NCPA, 2006a). Cottonseed oil intended for human consumption is highly purified/refined. The purification process substantially reduces the content of natural toxicants such as gossypol and cyclopropenoid fatty acids (FDA/CFSAN, 2002). The refined cottonseed oil is used as frying oil, salad and cooking oil, and in various foods including mayonnaise, salad dressing, shortening, and margarine (FDA/CFSAN, 2002).

Crude oil is inedible due to the presence of gossypol, while refined oil is edible as the gossypol has been denatured during the refining processes. Limited quantities of crude oil are used in the production of inedible products. Usually, these consist of off-grade oil or of the soapstock. Both crude oil and soapstock (that have already been minimally refined as described above) are used to produce fatty acids which in turn enter livestock feed and a wide range of industrial products such as soaps, emulsifiers, pharmaceuticals, insecticides, fungicides, cosmetics, rubber, plastics, and finishes for leather, paper, and textiles (NCPA, 2006b).

Expression of fusion proteins is not expected to occur in MON531. Analyses of the junction sequences of the DNA insert in MON531 do no indicate the expression of potential fusion proteins. Bioinformatic analyses of the 5' and 3' insert to plant junctions in MON531 provide support to this statement and were performed to assess the potential of toxicity, allergenicity or pharmacological activity of putative peptides encoded by the 5' and 3' junctions between the insert and the cotton genomic DNA (Hileman et al., 2001). The results of the bioinformatic analyses demonstrated that in the highly unlikely event that any of the junction polypetides were translated, they do not share a sufficient degree of sequence similarity or identity to indicate that they are potentially toxic, allergenic or have other health implications.

The scope of the current renewal application covers food additives, feed materials and feed additives produced from MON531 cottonseed. In support of our notification for MON531 under Regulation (EC) No 258/97, it has been demonstrated that there is no detectable level of protein in the primary product

for human consumption (i.e. refined cottonseed oil) produced from either biotechnology-derived or conventional cottonseeds (Berberich et al., 1993d; Goodman et al., 2001). Similarly, other highly processed foods produced from cotton would not be expected to contain detectable levels of protein. Therefore, the following information related to the assessment of allergenicity of the newly expressed protein can be considered as mainly informative.

The strategies employed to assess the allergenic risk focus on characterization of the source of the recombinant protein, the potential of the newly expressed protein to induce sensitization or to elicit allergenic reactions in already sensitized persons and whether the transformation may have altered the allergenic properties of the modified food. A weight of evidence approach is followed, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity.
Cottonseed oil and processed cotton linters are the primary cotton products used for human food (NCPA, 2006b). Analysis of cottonseed oil and processed cotton linters derived from both the Coker 312 control and MON531 confirmed that there is no detectable protein in either cottonseed oil (Fuchs, 1994) or processed cotton linters (Sims et al., 1995). Therefore, there will be no significant human consumption of the Cry1Ac and NPTII proteins present in insect-protected cotton varieties. Furthermore, direct food challenge of individuals allergic to proteins contained in the meal derived from oilseed crops (e.g., soybean, peanut, and sunflower) with the oil from these respective crops has established that refined oil does not elicit an allergenic response (Bush et al., 1985; Halsey et al., 1986; Taylor et al., 1981). This is consistent with the lack of detectable protein in the oil (Tattrie and Yaguchi, 1973). Based on this information, we conclude that there will be no significant human consumption of these proteins from cotton foods and food ingredients and that insect-protected cotton varieties pose no significant allergenic concerns.

If the Cry1Ac and NPTII proteins introduced into MON531 were consumed, this would not raise safety concerns, as these proteins have a long history of safe use and do not share the biochemical properties common to known allergenic proteins. The Cry1Ac protein expressed in MON531 is comparable to the Cry1Ac protein contained in microbial Bacillus thuringiensis subsp. kurstaki formulations, which have been available commercially and have been used safely for almost 30 years (Keck, P., Fuchs, R., Ream, J., 1994; Luthy et al., 1982).

These microbial formulations have been used on a wide variety of crops, including fresh produce such as lettuce and tomato, with no reported allergenic responses, establishing a sound basis for the lack of allergenic concern for the B.t.k. HD-73 protein. The U.S. EPA issued an exemption from the requirement of a tolerance for the Cry1Ac protein on August 31, 1995, and in doing so concluded there were no demonstrable grounds for allergenic concerns (US EPA, 1995). The NPTII protein was approved by the FDA as a processing aid food additive for tomato, cotton, and canola (FDA, 1994), and exempted from the requirement of a tolerance as a pesticidal inert ingredient by the EPA (EPA, 1994). These approvals included an assessment of potential allergenic affects for the NPTII protein, and concluded there were no significant concerns. Although large quantities of a vast variety of proteins are consumed in diets each day, rarely do any of these tens of thousands of proteins elicit an allergenic response (Taylor et al., 1992). Although there are no predictive bioassays available to assess the allergenic potential of proteins (FDA, 1992), the biochemical profiles of the Cry1Ac and NPTII proteins provides a basis for allergenic assessment when compared with known protein allergens. Thus, important considerations contributing to the allergenicity of proteins ingested orally include exposure and an assessment of the factors that contribute to exposure, such as stability to digestion, prevalence in the food, and consumption pattern (amount) for the specific food (Kimber et

al., 1999; Metcalfe et al., 1996). Additionally, protein allergens must be stable to the peptic and tryptic digestion and the acid conditions of the digestive system if they are to reach and pass through the intestinal mucosa to elicit an allergenic response. Another significant factor contributing to the allergenicity of proteins is their high concentration in foods that elicit an allergenic response (Taylor et al., 1987; Taylor, 1992; Taylor et al., 1992).
Although the full length Cry1Ac protein is proteolytically cleaved to a 67 kd protein that fits within the size range of known allergens, neither of these forms of the Cry1Ac protein possess the characteristics common to protein allergens. Similarly, the NPTII protein does not possess characteristics of common allergens. The biological activity of the Cry1Ac and NPTII proteins is lost upon the processing/toasting procedure used to remove cottonseed oil from MON531 (Fuchs, 1994). Western blot analysis of the processed cottonseed meal showed no detectable levels of the Cry1Ac protein in MON531; however, a portion of the NPTII protein (< 4% of the total NPTII protein in raw cottonseed meal) was still present in the processed material. These data indicate that the tertiary structure was altered and the proteins were converted to non-functional, denatured molecules during the processing procedure, as expected, since proteins are typically labile to high temperatures.
More importantly, the Cry1Ac and NPTII proteins were shown to be very labile to digestion by the proteases present in the mammalian digestive system, minimizing any potential for this protein to be absorbed by the intestinal mucosa, if consumed. In vitro simulated mammalian gastric and intestinal systems digestive mixtures were established and used to assess the susceptibility of the Cry1Ac and NPTII proteins to proteolytic digestion. The method of preparation of the simulated digestive solutions used is described in the United States Pharmacopeia (U.S. Pharmacopeia, 1990), a frequently cited reference for in vitro digestion. In vitro studies with simulated digestive solutions are widely used as models of animal digestion. They have been used to investigate the digestibility of plant proteins (Marquez and Lajolo, 1981; Nielsen, 1988), animal proteins (Zikakis et al., 1977), and food additives (Tilch and Elias, 1984) to assess protein quality (Akeson and Stahmann, 1964) to study digestion in pigs and poultry (Fuller, 1991) to measure tablet dissolution rates to monitor biodegradation for pharmaceutical applications (Alam et al., 1980) and to investigate the controlled-release of experimental pharmaceuticals (Doherty et al., 1991).

The data from the simulated digestion experiments demonstrated a half-life for Cry1Ac protein of less than 30 sec in the gastric system (Ream, 1994). As expected, in the intestinal system, the Cry1Ac protein was rapidly converted to the trypsin-resistant core, which was not further degraded. In similar experiments, the NPTII protein readily degraded in both simulated gastric and intestinal fluids with halflives of less than ten sec and between two and five min, respectively (Ream, 1993). To put the rapid degradation of these proteins in the simulated gastric system into perspective, solid food has been estimated to empty from the human stomach by about 50% in two h, while liquid empties 50% in approximately 25 min (Sleisinger and Fordtran, 1989). Therefore, any Cry1Ac or NPTII protein consumed would be rapidly degraded in the gastric system. It is also important to establish that the Cry1Ac and NPTII proteins do not represent previously described allergens, and further, do not share potentially immunologically relevant epitopes (amino acid sequences recognized by IgEs). Updated bioinformatic analyses have been performed on Cry1Ac and NPTII proteins expressed in MON 531 and are presented in Annex 3.5 "Updated toxicity and allergenicity data" of the "Specific information" in this renewal application.
Bacillus thuringiensis and its formulations used as microbial pesticides have not been described as sensitizing allergens, including through oral exposure (McClintock et al., 1995). Thus there is no apparent history of allergy associated with crystal proteins from Bacillus thuringiensis. Therefore, the potential allergenicity of the Cry1Ac and NPTII proteins has been evaluated and it is concluded that the proteins do not pose significant allergenic concerns.

MON531 is substantially equivalent to conventional cotton (Section D.7.1). Furthermore, it has been demonstrated previously that cottonseed oil does not contain detectable level of protein. In addition, studies of the introduced proteins in MON531 do not reveal any allergenic potential. In conclusion, the use of food or feed products produced from MON531 is unlikely to lead to an increased risk for allergenic reaction compared to the equivalent range of food and feed uses from conventional cotton.

Comments

Data are provided demonstrating that the Cry1Ac protein and the NPTII protein do not fulfill the classical criteria of allergens and it is stated that these proteins will not be present in cotton oil. Nevertheless the possible contamination of proteins in plant derived oils is dependent on the degree of refining and traces of proteins can be found in some plant derived oil (eg peanut oil) leading to symptoms is sensitized persons. Allergens can elicit clinical reaction even in very minute amounts, not detectable with commonly used methods for protein determination (Olszewski et al, 1998).

Furthermore, digestion of proteins destroys most of the conformational epîtopes but not all linear epitopes (Untersmayr & Jensen-Jarolim, 2008).

It would be advisable to add these caveats to the conclusions about allergenicity.

Comment 2

No questions

Comment 3

Assessment of the allergenicity of the newly expressed proteins.

The reviewer agrees that the newly introduced proteins are not likely to be allergenic.
However, although Cry1Ac is not likely to be an allergen, it should be emphasized that Cry1Ac has been proposed as an adjuvant for vaccines (Vásquez et al. Scand J Immunol 1999, 49:578-84; Vásquez-Padrón et al. Life Sci 1999, 64:1897-912; Moreno-Fiéros et al. Scand J Immunol 2003,57:45-55; Esquivel-Pérez et al. Viral Immunol 2005, 18:695-708), which means that this protein is able to enhance the immune responses against antigens that are co-administered. This is not uncommon for a bacterial protein. The consequence of the presence of such immuno-stimulant in a plant destined to human consumption is not known. Particularly the adjuvant effect via intestinal route is poorly documented. It is not known whether the presence of Cry1Ac in cottonseeds might elicit sensitization against the other cotton proteins upon ingestion. However, given the scope of the application (food produced from GM plants or containing ingredients produced from GM plants), the level of expression in seeds and the general usage of cotton in the food industry (refined oil product), such adjuvant effect is not likely to be an issue. Nevertheless, it might be relevant to study in mice the immune responses against cotton proteins when the animals are fed MON531 cottonseeds.
At several occasions, the applicant claimed that protein levels in refined oil are so low that they do not represent allergy risk for this sole reason. However, protein levels in oils and their possible role in allergy are controversial issues, and protein levels may be subjected to batch-to-batch variations.

Assessment of the allergenicity of the whole GM plant or crop.

The applicant did not evaluate the potential allergenicity of MON531 cottonseeds, compared to their traditional counterpart. The reviewer agrees that cottonseed allergy is not a major issue and that no major allergen of cottonseed has been characterized. However, seeds of all kinds may contain potent allergens, like 2S storage proteins and vicillins. Because the introduction of the new traits might influence the expression levels of other proteins of the host plant by a cascade effect, it might be relevant to evaluate the content of 2S storage protein and vicillin in the MON531cottonseeds.

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)

Comment 1

No questions

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

NOT APPLICABLE

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

NOT APPLICABLE

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

NOT APPLICABLE

D.11. ENVIRONMENTAL MONITORING PLAN

NOT APPLICABLE

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