Bioveiligheidsraad
Conseil de Biosécurité

10/02/2012

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2012_0217

Title: Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/RX-MON1445 from Monsanto under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/RX-MON1445 was submitted by Monsanto on 29 June 2007 for renewal of the authorisation of (1) foods produced from genetically modified cotton MON 1445 (contonseed oil and food additives) and (2) feed produced from genetically modified cotton MON 1445 (feed materials and feed additives) within the framework of Regulation (EC) No. 1829/2003[1].
Cotton MON 1445 expresses the CP4 EPSPS protein conferring tolerance to glyphosate, and also carries genes coding for neomycin phosphotransferase type II (NPTII) and 3′(9)-O-nucleotidyltransferase (AAD, not expressed in MON 1445), which were used as antibiotic resistance marker genes during product development.

The application was officially acknowledged by EFSA on 3 July 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). Eight experts answered positively to this request, and formulated a number of comments to the dossier, which were edited by the coordinator. See Annex I for an overview of all the comments and for the list of comments placed on the EFSAnet on 3 October 2008.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 3 November 2011 (EFSA Journal, 2011;9(12):2479)[2], and published on 16 December 2011 together with the responses from the EFSA GMO Panel to comments submitted by the Member States during the three-month consultation period.

On 21 December 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/2479.htm>

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

This GM cotton contains the antibiotic resistance marker genes *nptII* and *AAD* (the latter being not expressed in the plant). The EFSA scientific opinion contains a detailed evaluation of risk assessment of the presence of these ARM genes in particular with regards to the potential risk arising from their horizontal gene transfer (HGT) to bacteria. Considering the low level of exposure and the expected low frequency of gene transfer from MON 1445 to bacteria compared to that between bacteria, and based on the EFSA Statement on the use of these two antibiotic resistance genes as marker genes in genetically modified plants, the contribution of HGT to the environmental prevalence of ARM genes is considered negligible.
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 tocopherol (Vitamin E) in cottonseed (tocopherol levels were measured only in oil), nor the analysis of Calcium and Phosphorus levels in meal as recommended by the OECD consensus document on compositional considerations for new varieties of cotton[4].

In addition, the compositional analysis showed that the level of the natural toxicant gossypol (total and free) was significantly higher in cotton MON 1445 than in the comparator in the material harvested from field trials performed in 1993 and 1994. The Biosafety Advisory Council agrees that the toxicological relevance of these statistically significant differences in gossypol levels could be considered negligible in the context of the intended use, as no gossypol (total and free) could be detected in the refined oil and no free gossypol in the toasted meal derived from the GM cotton. Nevertheless, given the toxic nature of gossypol, the Biosafety Council is of the view that these differences should trigger additional analysis to unequivocally conclude about the safety of all products of this GM cotton for which authorization is requested.

[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 performed.
[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

3.2. Assessment of toxicity

The compositional analysis led to uncertainties as regards the compositional equivalence between cotton MON 1445 and its comparator taking into account the background range of variation in commercial cotton varieties.
The Biosafety Advisory Council is therefore of the opinion that the information provided is not sufficient to conclude about the toxicological safety of this GMO and that further toxicological assessment should be performed.

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

In the compositional analysis, statistically relevant differences in composition were found between cotton MON 1445 and its conventional counterpart. In addition, the compositional analysis was not fully performed according the OECD recommendations.
The Biosafety Advisory Council notes that two feeding studies were performed, respectively on catfish and in Sprague-Dawley rats. These studies did not reveal any biologically significant differences in the measured parameters. However, the data on body weight in the study performed on catfish were not supported by an appropriate number of replications. Moreover, a 42-day poultry feeding study should have been performed to comply with the EFSA Guidance for risk assessment of food and feed from genetically modified plants[5].
Therefore, the Biosafety Advisory Council is of the opinion that the information provided is not appropriate to demonstrate the nutritional equivalence of this 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.

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,

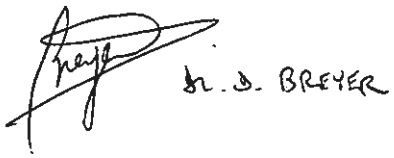
The Biosafety Advisory Council is of the opinion that
- the applicant did not follow completely the OECD recommendation on the comparative compositional analysis and did not argue why not;
- relevant statistical differences were observed in the compositional analysis concerning the level of the natural toxicant gossypol, raising uncertainties as regards the toxicological safety of this GM cotton;
- as the compositional analysis does not take away all concerns and according to EFSA guidance for risk assessment of food and feed from genetically modified plants, further

[5] EFSA Journal 2011; 9(5):2150

nutritional and toxicological assessment should be performed to exclude any potential risk for human and animal health.

Based on the above-mentioned considerations, the Biosafety Advisory Council gives a negative advice regarding the health safety of this event.

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



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

Annex I: Full comments of experts in charge of evaluating application EFSA/GMO/RX-MON1445 and comments submitted on the EFSAnet (ref. BAC_2008_820)

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Conseil de Biosécurité

Secretariaat
Secrétariat

N./réf. : WIV-ISP/BAC_2008_820
Email. : bac@sbb.ihe.be

03-10-2008

Compilation of comments of experts in charge of evaluating the application EFSA/ EFSA/GMO/RX-MON1445
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 22 August 2008
Coordinator: Prof. Philippe Baret
Experts: Pascal Cadot (Consultant), Rony Geers (KUL), Jean-Luc Hofs (FUSAGx), André Huyghebaert (UGent), Peter Smet (Consultant), Frank Van Breusegem (VIB), Jan Van Doorsselaere (KH Zuid-West Vlaanderen), Johan Van Waes (ILVO).
Domains of expertise of experts involved: Genetics, Genetic engineering, Molecular characterisation, Transgene expression, Bioinformatics, Ecotoxicology, Agronomy, Breeding, Ecology, Biodiversity, Herbicide tolerance, Cotton, Human nutrition, Animal nutrition, Analysis of food/feed, Industrial processing, Toxicology, Immunology, Alimentary allergology, Statistics
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

INTRODUCTION

Dossier **EFSA/GMO/RX-MON1445** concerns an application of the company **Monsanto** for the renewal of the marketing authorisation of the genetically modified **cotton MON1445** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 03 July 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

According to the dossier the scope of application does not include the authorization for the cultivation of MON 1445 cotton seed products in the EU. It can however be worthwhile to give some remarks on the different topics, dealing with cultivation and survivability of seeds, in the case that the applicant should ask in the near future for an extension for the scope of cultivation, especially for cultivation in some southern European countries.
So as agronomical expert I will also give some comments in this questionnaire, related to cultivation and the environmental aspect.
Remark SBB and coordinator:
This comment has not been sent to EFSA because it is not relevant given the scope of the application. Same comment has not been forwarded to EFSA in other similar dossiers.

Comment 2

Information satisfactory.

Comment 3

No comments

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

Comments/Questions of the expert(s)

Comment 1

Under "3. Survivability – specific factors affecting survivability" it is mentioned that cotton does not possess any of the attributes associated with long term survivability such as seed dormancy,.. Furthermore it is mentioned that it is highly unlikely that cottonseed would overwinter and germinate the following spring, unless in regions with mild and cold winters such as in Spain and Greece. My question is : are there data available of overwintering of seed of cotton in those regions? And if yes could the seedlings be controlled by the use of herbicides, such as glufosinate and paraquat?
Remark SBB and coordinator:
The comment above has already been placed on the EFSAnet for application 42. Comments on the survivability of cotton seeds have also been placed on the EFSAnet for applications 41 and 51. However, the scope of all these applications also covered import and processing (and thus accidental spillage during transport had to be envisaged), which is not the case for the current application.
In consequence, we think that this comment is out of scope in this specific case.

Moreover, the same comment made for application RX-MON15985xMON1445 has not been forwarded to EFSA.
Accordingly, this comment has not been sent to EFSA.

Comment 2

Information satisfactory.

Comment 3

No comments

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Comment 1

Information satisfactory and sufficient as previous EC notifications are taken in to account.

Comment 2

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 comment

Comment 2

Information satisfactory. Since the former application, there is no new significant scientific result or information calling these characteristics into question.

Comment 3

No comments

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

Comments/Questions of the expert(s)

Comment 1

The quality of some of the Southern blots is poor.
In figures 6 and figure 9 (panel A) bands in the controls are not visible.

The quality of figure 12 is insufficient and it cannot be concluded that MON1445 contains one T-DNA copy (for instance I do not see a specific band hybridizing with the SpeI digested genomic DNA).
The segregation data presented in section 5 of the insert in the progeny are adequate and demonstrate the presence of 1 T-DNA copy. The southern blot in figure 19 is good and also shows that 1 T-DNA copy is present in the R3 and R5 generations.

P.43: Where do the 5' and 3' flanking primers come from?
P.42: How was it shown that 67 bp were deleted at the T-DNA junction?

Comment 2

Information satisfactory

Comment 3

No comments

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1

No comment

Comment 2

Information satisfactory and complete. Since the former application, there is no new significant scientific result or information calling the CP4EPSPS expression issues into question.

Comment 3

No comments

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

Comments/Questions of the expert(s)

Comment 1

Remarks concerning the survivability of seeds of cotton. In the dossier it is mentioned that seed of cotton is known to be a weak competitor in the wild, which can not survive outside cultivation without human intervention. Furthermore the applicant mentioned that field observations have demonstrated that MON1445 has not been altered in its survivability when compared to conventional cotton. My question is : are there real data to prove that there is in not any case survival of seed, even after optimal conditions in the field during winter in southern European countries, were observed.

Remark SBB and coordinator:

The comment above has already been placed on the EFSAnet for application 42. Comments on the survivability of cotton seeds have also been placed on the EFSAnet for applications 41 and 51. However, the scope of all these applications also covered import and processing (and thus accidental spillage during transport had to be envisaged), which is not the case for the current application.

The comment above has also been forwarded to EFSA for applications RX-MON15985 and RX-MON15985xMON1445 (although these applications did not cover import and processing).

Nevertheless, we are of the view that this comment is out of scope for the current application and accordingly, it has not been sent to EFSA.

Comment 2

No comment

Comment 3

No Comment and not very applicable.

Comment 4

No comments

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

Comments/Questions of the expert(s)

Comment 1

No comment

Comment 2

No new significant elements have been published since the first application. Information satisfactory.

Comment 3

No comments

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

Comments/Questions of the expert(s)

Comment 1

No comment and not very applicable.

Comment 2

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

In this chapter it is mentioned that MON1445 was also compared to other commercial conventional cotton varieties. What does it mean? The MON1445 is tolerant to glyphosate. So I think it is not possible to compare with commercial conventional varieties, unless they are also tolerant to glyphosate (= are also genetically modified). My question is : Is MON1445 compared to other genetically modified varieties or only to conventional varieties and in the last case was the herbicide tolerance taken into account in this comparison?

Remark SBB and coordinator:

For consistency with comments placed for previous applications (dossiers 41, 42, RX-MON15985 and RX-MON15985xMON1445) we forwarded the following comment to EFSA:

"In this chapter it is mentioned that MON1445 was compared to other commercial conventional cotton varieties. The choice of these varieties should be better documented and motivated (other genetically modified varieties or only conventional varieties and in the last case was the herbicide tolerance taken into account in this comparison?)."

Comment 2

The comparative assessment should also address the herbicide use on the transgenic variety (and not the transgenic cultivar without herbicide treatment only) in order to detect possible increase of herbicide residue in the plants parts (Glyphosate, AMPA, POEA…).

Remark SBB and coordinator:

This comment has been taken on board with the proposal under "Comment 1".

Comment 3

MON1445 was compared with Coker 312, the conventional variety. Both strains were grown at six locations in the US, representative for cottonseed production.
Proximate analysis covers % fat, ash, protein, carbohydrates, moisture and Calories per 100g.
No statistical difference was found for % fat, ash, moisture and Calories content per 100g.
A statistical difference was observed for % protein between the two strains

Remark coordinator:

The sentences above imply that the substantial equivalence is not demonstrated. They have been forwarded to EFSA.

The values obtained are however within the ranges found in literature. The applicant considers this difference as not relevant. The carbohydrate level was calculated by difference so the same conclusion applies as for protein.

The amino acid analysis covers a broad range of constituents, including the essential amino acids and the sulphur containing acids. Particular attention was given to aromatic amino acids. Values obtained are within the range of literature data.

The fatty acid composition has been studied in detail. No differences have been found between both cottonseeds. The analysis included the usual fatty acids and the important cyclopropenoid acids: malvalic, sterculic and dihydrosterculic acid.

Gossypol, a terpenoid structure present in cottonseed has been investigated. Gossypol is an important constituent as it has toxic properties, when present in human food and animal feed. The substance is removed or inactivated during processing. Somewhat higher levels of gossypol have been found in MON1445 than in the conventional variety Coker 312. However a broad range of data has been found in literature. The applicant states that the differences in gossypol amounts found are not meaningful. I agree with this conclusion.

In addition the level of allelochemicals present in leaves has been assessed. No differences in levels of flavonoids, tannins and anthocyanin have been found.

Attention has also been paid to the sensitivity to aflatoxin formation. This is an important aspect as cottonseed is one of the commodities most commonly contaminated. No aflatoxins B1, B2, G1 and G2 have been detected at the 1 ppb level.

The applicant concludes from the 1993 analysis that MON1445 is compositionally equivalent to the parental Coker 312.

Samples from both strains have also been collected and analyzed during the 1994 season.

Results from proximate analysis are much in line with the previous results. The same applies for MON1445 treated with Roundup.
No statistical difference was found in the amino acid composition.
Within the fatty acid composition some differences were found. Values obtained were within the range of literature data.

Gossypol was studied in somewhat more detail as free gossypol was assessed in addition to total gossypol. In line with previous results, values for total gossypol were higher in MON1445, treated with Roundup and untreated, than in Coker 312.
Remark coordinator:
How does the notifier explain this difference ? This comment has been forwarded to EFSA.

Once again data are within the range of literature values.

Further information was obtained by analysis of seeds from the 1998 and 1999 trials. Data cover proximates, amino acids, fatty acids, anti-nutrients like gossypol and minerals like Ca, Cu, Fe, Mg, Mn, P, K, Na and Zn.

The applicant concludes that MON1445 is comparable to the control line in terms of proximates, nutrients and anti-nutrients.
I agree with this conclusion taking into account that the dossier was already subject to a previous review.

However I have some remarks in view of the information present in more recent dossiers:
- there is no information available on dietary fibre, only about crude fibre, as mentioned before
- limited data are present about minor constituents like vitamins; however vit E has been studied in the framework of processing,
- specific anti-nutrients, such as gossypol and cyclopropenoid acids, and contaminants, such as aflatoxins, have been studied in detail.

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

In this chapter it is mentioned that MON1445 was compared with the control Coker 312 in 1993 and 1994. My question is if in these trials the resistance for glyphosate is also tested, in relation to yield.

Comment 2

Did the trial include a treatment made of MON1445 cotton plants sprayed with Glyphosate at the recommended rate and dose? As it is not indicated in the description of the experiment, we assume that it has not been performed.

Comment 3

No particular comments.

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

The importance of the non target approaches as functional genomics, proteomics, or metabolomics (see Molnar, 2005; Yonekura-Sakakibara and Saito, 2006) should be highlighted. Given the recent advances in this field such information should be presented, even partially, in an application renewal

Remark SBB:

The same comment has been forwarded to EFSA for applications RX-MON15985 and RX-MON15985xMON1445.

It should be noted however that EFSA already took a position on this issue in its recent paper (Food and Chemical Toxicology 46 (2008) S2-S70), EFSA states (on p. S56): "Regarding the analytical detection of unintended effects, profiling technologies such as transcriptomics, proteomics and metabolomics are promising tools, which will broaden the spectrum of detectable compounds and supplement current targeted analytical approaches. These technologies are still under development, and need validation before they can be used for routine safety assessment purposes."

Comment 2

Compositional studies are based on the current approach in 1993 and 1994. Taking into account the OECD recommendations additional information on vit E was obtained.
Additional information was submitted in the context of the application of the combined-trait product MON531 x MON1445.

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

Information and comments are satisfactory.

Comment 2

No further comments.

D.7.5 Product specification

Comments/Questions of the expert(s)

Comment 1

No comment

Comment 2

No comment.

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1

No comment for the introduced gene or CP4 EPSPS enzyme.
Does seed processing into linter, oil and cake leads to Glyphosate, AMPA and POEA residues degradation into non toxic compounds?
Remark coordinator:
Comment not relevant to biosafety.

Comment 2

Cottonseed from the 1993 trials was processed into full fat flour, toasted meal and oil according to traditional processing methods. Full fat flour and toasted meal serve as animal feed whereas oil is used after refining as a human food.
It was demonstrated that any gossypol left over in the crude oil was removed during refining, as could be expected.
Alpha-tocopherol (vitamin E) levels were assessed in the crude oil from both types of cottonseed. The levels were similar in both lines. During refining tocopherols are lost depending on the refining conditions.
The fatty acid profiles of the refined oil of both lines was similar.
Particular attention was given to the fate of cyclopropenoid acids during refining. It was demonstrated that the level was reduced to a minimum during refining.
No further remarks.

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

Information correct.

Comment 2

No further comment.

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

Three studies are available, i.e. Naylor (1992) with rats, Naylor (1993) with mice and Jackson et al. (1994) with catfish. Based on the guidelines to provide sufficient power in the statistical analysis, e.g. Berndtson (Berndtson, W. E. 1991. A simple, rapid and reliable method for selecting or assessing the number of replicates for animal experiments. J. Anim. Sci. 69:67–76), only the data on body weight of rats (Naylor, 1992) are supported by an appropriate number of replications.
Remark coordinator:
We can insist on the fact that this lack of power is an important issue, as stated in the recent EFSA draft document on "Statistical considerations for the safety evaluation of GMOs".

Comment 2

5a) CP4 EPSPS protein measured in MON 1445 (additional data from 2001).

Tissue	**ng/mg Tissue Fresh Weight**		**Standard deviation**
	Mean	**Range**	
Leaf	52	35-73	15
Seed	110	100-170	6.8

Tissue	**ng/mg Tissue Dry Weight**		**Standard deviation**
	Mean	**Range**	
Leaf	310	170-440	110
Seed	120	110-130	7

5b) NPTII protein measured in MON 1445.

Tissue	**ng/mg Tissue Fresh Weight**		**Standard deviation**
	Mean	**Range**	
Leaf	23	19-36	7.1
Seed	16	13-17	2.0

Tissue	ng/mg Tissue Dry Weight		Standard deviation
	Mean	Range	
Leaf	130	100-200	39
Seed	17	14-19	2.2

Comment 3

The use of herbicide "over-the-top" application linked with the genetic modification should be assessed for Glyphosate residues and AMPA metabolite, as well as the surfactant residues. The applicant should demonstrate (or discuss) the innocuousness of the specific herbicide tolerant crop management on human and animal health. The introduction of RR plants in the fields increased significantly the quantities of RoundUp ® herbicide on the plant (Benbrook, 2004; case of soybean: Bonny, 2008) compared with the pesticide drift occurring in conventional directed application.

Remark SBB and coordinator:

This comment relates to the current discussion regarding the interplay between GMO regulations and the regulation governing pesticide registration in Europe (Directive 91/414/EC). This issue is currently under consideration by EFSA (self-tasking activity) and should led very soon to the publication of a draft guidance which will be open for public consultation.

However, it was considered interesting to forward this comment to EFSA in order to get their preliminary views on this issue.

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

a) Degradation of the CP4 EPSPS protein in simulated gastric fluid (Harrison et al., 1996).

A half-life of less than 15 seconds in the gastric system.

b) Degradation of the CP4 EPSPS protein in simulated intestinal fluid (Harrison et al., 1996).

A half-life of less than 10 minutes in the intestinal system.

c) CP4 EPSPS: Acute Oral Toxicity Study in Mice (Harrison et al., 1996).

There were no adverse effects in mice administered CP4 EPSPS protein by oral gavage at dosages up to 572 mg/kg.

d) CP4 EPSPS protein: Sequence homology with known toxins (McCoy and Silvanovich, 2003).

The comparison was performed with the allergen (AD4), toxin (TOXIN5), and public domain (ALLPEPTIDES) database sequences using bioinformatic tools.

No biologically relevant structural similarities to allergens, toxins, or pharmacologically active proteins were observed for the CP4 EPSPS protein sequence.

e) Degradation of the NPTII protein in simulated gastric fluid (Ream, 1993; Fuchs et al., 1993c).

A half-life of less than 10 seconds in the gastric system.

f) Degradation of the NPTII protein in simulated intestinal fluid (Ream, 1993; Fuchs et al., 1993c).

A half-life between 2 and 5 minutes in the intestinal system.

g) NPTII: Acute Oral Toxicity Study in Mice (Naylor, 1992).

There were no adverse effects in mice administered NPTII protein by oral gavage at a dosage of 5000 mg/kg.

7h) NPTII protein: Sequence homology with known toxins (Hileman and Astwood, 2000).

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 NPTII protein was compared with this toxin sequence database using the FASTA sequence alignment tool. In addition, the amino acid sequence of the NPTII protein was compared with all protein sequences in publicly available genetic databases to screen for structural similarity to pharmacologically active proteins. NPTII shared sequence similarities to homologous aminoglycoside modifying enzymes, as expected.
No other significant structural homology was observed.

Conclusion: No further testing is needed

Comment 2

Information satisfactory as former studies were completed with bioinformatics results.

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Comment 1

Information is correct. Since the former application, there is no new significant scientific result or information calling the presence of new plant constituant into question.

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1

Information is correct.

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

a) 42-day poultry feeding study (author)

Not performed
Remark SBB and coordinator:
For consistency with applications 41, 42, RX-MON15985 and RX-MON15985xMON1445, and with a previous remark on this dossier, the following comment was forwarded to EFSA:
"A 42-day poultry feeding study is not provided in the dossier. We assume that this is based on the fact that the comparative analysis between the GM crop and the traditionally grown crop with respect to compositional characteristics has been carried out appropriately and that no statistically significant differences in the composition of the GM plant compared to its non-GM comparator have been identified. However, this assumption can be questioned since we pointed out that a statistical difference was observed for % protein (see our comment under D7.1 – Comparative assessment). Moreover, we would like to remind that some Belgian experts have already expressed concerns about the fact that the compositional analysis is sufficient per se to drawn general conclusions concerning the safety of the whole GMO."

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

Not performed

c) 10-Week catfish feeding study (Jackson, 1994)

Weight gain, feed per fish, feed conversion ratios, and survival were not significantly different between the test and control lines during the study. The body composition data were typical of that observed in numerous other studies with catfish of the size used in the study. The differences in fat due to dietary treatment (12.3% compared to fish fed the control diet 14.6% fat on a dry weight basis) seem to be small.

Conclusion: No further testing is needed

Comment 2

Information correct and satisfactory.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

Assessment of the allergenicity of the newly expressed proteins.
The reviewer agrees that the newly introduced proteins (CP4 EPSPS and NPTII) are not likely to be allergenic.
In addition, 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), the presence of these proteins is not likely to be an allergenic issue.
For information: 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 MON1445 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. Nevertheless, 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 MON1445 cottonseeds, certainly in the hypothesis that abnormal higher protein levels appear in a batch of the final product for human consumption.
Remark SBB:
The same kind of comment has been forwarded to EFSA for GM cotton applications 41, 42, 51, RX-MON15985, RX-MON15985xMON1445 and RX-MON531.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1

It is noted that the introduced trait of glyphosate, which is an agronomic trait, is not intended to change any nutritional aspects of this cotton. Can this be proved by data?
Remark SBB and coordinator:
As for application RX-MON15985xMON1445, this comment was not forwarded to EFSA.

Comment 2

Information correct.

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

Comments/Questions of the expert(s)

Comment 1

Post-market monitoring should take herbicide residues into account in order to clear any doubt related to the impact of RR plants agricultural practices on food/feed safety.

Remark SBB and coordinator:

This comment relates to the current discussion regarding the interplay between GMO regulations and the regulation governing pesticide registration in Europe (Directive 91/414/EC). This issue is currently under consideration by EFSA (self-tasking activity) and should led very soon to the publication of a draft guidance which will be open for public consultation.

However, it was considered interesting to forward this comment to EFSA in order to get their preliminary views on this issue.

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

Comments/Questions of the expert(s)

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

D.9.1. Persistence and invasiveness

Comments/Questions of the expert(s)

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

D.9.6 Effects on human health

Comments/Questions of the expert(s)

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

References

Berndtson, W. E. (1991). A simple, rapid and reliable method for selecting or assessing the number of replicates for animal experiments. J. Anim. Sci. 69:67–76.

Remark coordinator:

Interesting reference to join with comment on toxicity.