Bioveiligheidsraad
Conseil de Biosécurité

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Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2014_0733

Title: Advice of the Belgian Biosafety Advisory Council on applications EFSA/GMO/UK/2008/57 and EFSA/GMO/RX-MON15985 from Monsanto under Regulation (EC) No. 1829/2003

Context

The application EFSA-GMO-RX-MON15985 was submitted by Monsanto on 29 June 2007 within the framework of Regulation (EC) No. 1829/2003[1] for renewal of the authorisation of food additives and feed (feed materials and feed additives) produced from insect-resistant genetically modified (GM) cotton MON 15985.

Cotton MON 15985 was obtained by the transformation of GM cotton MON 531 with a DNA fragment carrying two expression cassettes: *cry2Ab2* and *uidA*. Expression of the Cry2Ab2 protein confers resistance to the major lepidopteran cotton pests while the GUS E377K protein, produced by the uidA gene, was used as a histochemical marker during product development.
Cotton MON 531 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 (*aadA*, under the control of its bacterial promoter and therefore not expressed in MON 531), which were used as antibiotic resistance marker genes during product development.

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

On 22 May 2008, EFSA received application EFSA/GMO/UK/2008/57 submitted under Regulation (EC) No. 1829/2003 for the authorisation of cotton MON 15985 for import and processing, and for food and feed uses. It was officially acknowledged by EFSA on 20 August 2008.

On the same date EFSA started the formal three-month consultation period of the Member States. Within the framework of this consultation, the 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 SBB. Two experts answered positively to this request,

[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)

and formulated a number of comments to the dossier, which were edited by the coordinator. See Annex II for an overview of all the comments and for the list of comments actually placed on the EFSAnet on 20 November 2008. It was clearly stated at that time that comments provided by the BAC for application EFSA-GMO-RX-MON15985 were also valid for application EFSA/GMO/UK/2008/57.

Since both EFSA/GMO/UK/2008/57 and EFSA-GMO-RX-MON15985 cover cotton MON 15985, the EFSA GMO Panel adopted on 2 July 2014 a single scientific opinion, valid for both applications. This opinion was published on 28 July 2014 (EFSA Journal 2014;12(7):3770)[2] together with the responses from the EFSA GMO Panel to comments submitted by the Member States during the three-month consultation periods on application EFSA/GMO/UK/2008/57. The responses from the EFSA GMO Panel to comments submitted on application EFSA-GMO-RX-MON15985 were made available on 29 August 2014.
The EFSA opinion and the responses from the EFSA GMO Panel to comments submitted by the Member States on applications EFSA/GMO/UK/2008/57 and EFSA-GMO-RX-MON15985 were forwarded to the Belgian experts on 30 July and 29 August 2014 respectively. In both cases, the experts 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 Biosafety Advisory Council issues below a single comprehensive advice covering both applications EFSA/GMO/UK/2008/57 and EFSA-GMO-RX-MON15985. In delivering its advice the BAC considered in particular the following set of information:
- The EFSA opinion on these applications including the answers of the EFSA GMO Panel to comments submitted by the Member States;
- The comments formulated by the Belgian experts on both applications;
- The advice already published by the BAC on GM cotton MON 531[3]. On this GM event, the BAC did not identify any risk that the import and processing of this GM cotton could pose to the environment, but did not give an advice on the health safety of the GMO. The BAC was indeed 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.

Scientific evaluation

1. Environmental risk assessment

GM cotton MON 15985 contains the antibiotic resistance marker genes *nptII* and *aadA* (the latter being not expressed in the plant). EFSA provided 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 15985 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. Nevertheless, since alternatives to antibiotic resistance marker genes exist and in the context of the phasing out of such markers in GM plants foreseen by Directive 2001/18/EC, the Biosafety Advisory Council recommends that antibiotic resistance marker genes should not be used anymore in GM plants.
According to the Biosafety Advisory Council no major risks were identified concerning the European environment[4].

[2] See http://www.efsa.europa.eu/en/efsajournal/pub/3770.htm
[3] Advice of the Belgian Biosafety Advisory Council of 16 December 2011 on application EFSA/GMO/RX-MON531 from Monsanto under Regulation (EC) No. 1829/2003 (ref WIV-ISP/41/BAC/2012_0034)
[4] Since this application does not imply a cultivation of the GM crop in the EU, a full environmental assessment is not required in EFSA procedure and was not achieved.

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 Biosafety Advisory Council is of the opinion that GM cotton MON 15985 is compositionally equivalent to its conventional counterpart.

3.2. Assessment of toxicity

Cotton MON 15985 expresses four new proteins: Cry1Ac, NPTII, Cry2Ab2 and GUS E377K. Based on previous assessments of some of these proteins (Cry1Ac, NPTII, Cry2Ab2) and taking into account the information provided by the applicant, the Biosafety Advisory Council is of the opinion that in the context of its intended uses GM cotton MON 15985 does not raise safety concerns regarding toxicity.

3.3. Assessment of allergenicity

The Biosafety Advisory Council agrees with the EFSA GMO Panel that there are no indications that the newly expressed proteins in GM cotton MON 15985 may be allergenic under the intended conditions of use.
Since 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 GM cotton MON 15985 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 EFSA opinion, the answers of the EFSA GMO Panel to the questions raised by the Belgian experts, the answers of the applicant to the questions of the EFSA GMO Panel and considering the data presently available, the Biosafety Advisory Council is of the opinion that in the context of its intended uses, GM cotton MON 15985 is unlikely to pose any risk to human and animal health.

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

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

Prof. Maurice De Proft
President of the Belgian Biosafety Advisory Council

Annex I: Compilation of comments of experts in charge of evaluating application EFSA/GMO/RX-MON15985 and comments submitted on the EFSAnet (ref. WIV-ISP/BAC_2008_770)
Annex II: Compilation of comments of experts in charge of evaluating application EFSA/GMO/UK/2008/57 and comments submitted on the EFSAnet (ref. WIV-ISP/BAC_2008_844)

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Bioveiligheidsraad
Conseil de Biosécurité

18/06/2008

Secretariaat
Secrétariat

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Compilation of comments of experts in charge of evaluating the application EFSA/GMO/RX-MON15985 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 18 April 2008
Coordinator: Prof. Philippe Baret
Experts: Dr. Pascal Cadot (Consultant), Eddy Decuypere (KUL), Leo Fiems (ILVO), Jean-Luc Hofs (FUSAGx), Peter Smet (Consultant), Frank Van Breusegem (VIB), Jan Van Doorsselaere (KH Zuid-West Vlaanderen), Johan Van Waes (ILVO)
Domains of expertise of experts involved: Genome analysis, genetic engineering, molecular characterisation, bioinformatics, animal nutrition, biochemistry of food/feed, toxicology, immunology, alimentary allergology, agronomy, breeding, improvement of plants, ecology, ecotoxicology, biodiversity, herbicide tolerance, biosafety research, cotton
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

INTRODUCTION

Dossier **EFSA/GMO/RX-MON15985** concerns an application of the company **Monsanto** for the for the renewal of authorisation of the genetically modified **cotton MON15985** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 18 March 2008.
The scope of the application is:
☐ GM plants for food use
☐ Food containing or consisting of GM plants
☒ Food produced from GM plants or containing ingredients produced from GM plants
☐ GM plants for feed use
☒ Feed produced from GM plants
☐ Import and processing (Part C of Directive 2001/18/EC)
☐ Seeds and plant propagating material for cultivation in European Union (Part C of Directive 2001/18/EC)

Depending on their expertise, the experts were asked to evaluate the genetically modified plant considered in the application on its 1) molecular, 2) 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).
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 MON15985 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: Not relevant given the scope of the application (Food produced from GM plants or containing ingredients produced from GM plants; Feed produced from GM plants; But NO import, processing or cultivation)

Comment 2

MON15985 cotton has been introduced for commercial use for several years in some parts of the world. Up to now, there are no indications of adverse effects.
With regard to Cry1Ac and NPTII proteins, the safety assessments have been previously discussed in the notification pursuant to Regulation (EC) No 258/97.

Comment 3

No comments

Comment 4

Information provided is satisfactory.

Comment 5

Genetically fixed germplasm, homozygous for the inserted genetic elements in both MON531 and MON15947 is produced by traditional breeding processes.
MON531 expresses Cry1Ac insert protection protein and the NPT-II selectable marker protein, a neomycin phosphotransferase type II and a portion of the bleomycin binding protein.
MON15917 expresses Cry2 AB_2 insert protection protein and GUS proteins, a β-glucuronidase from E.Coli.
The combination of the genetic material responsible for the Cry 1Ac and Cry2 AB_2 production from MON531 and MON15947 respectively is known as MON15985.

Therefore the following proteins (Cry2 AB_2, Cry1 Ac, GUS, NPT-II and AAD, a 3' (9)-O-nucleotridyltransferase) in various tissues are compared between GM-cotton (MON15985) and control cotton, but also between MON15985 and MON531 cotton.

Comment 6

No comments

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

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

Information provided satisfactory, within the scope of the application renewal.
Nevertheless updated information should be included about pollen dispersal (Van Deynze et al., 2005; Llywellyn et al., 2007).

Comment 3

No comments

Comment 4

Under "3. Survivability – specific factors affecting survivability" it is mentioned 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?

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

Information sufficient when previous EC notifications are taken into account. Nevertheless it would be more relevant to include in this report a short version of the information related to MON531 event.

Comment 3

The uid-A gene cassette is expressing β-D-glucuronidase gene or GUS-proteins. This is important to consider since glucuronidation, addition of a glucuronide, is a process that increases solubility of compounds and hence plays an important role in the excretion of foreign substances from the body; the liver is the primary place of glucuronidation. It makes compounds less toxic and easier to excrete. Reversal of this process in the gut by internal glucuronidase activity may reverse this.

Comment 4

Page 14: As was mentioned by an expert in the evaluation of MON15985, the use of MON15947 is indeed confusing.
However on p75 of this dossier (RX-MON15985) it is clearly stated that the insert containing uidA en Cry2Ab2 is called MON15947 and that MON15985 is the result of retransformation of MON531 with this insert.
No further comments.
Remark SBB and coordinator:
In the frame of application 42 (Cotton MON88913xMON15985), the following request for clarification concerning the construct has been placed on the EFSAnet:
"The description of the MON15985 event is not completely clear. It is reported first that MON15985 was generated by transformation of MON531 with a vector containing the cry2Ab2 and uidA cassettes. However on p.18, another event (MON15947) is mentioned. Was MON15947 really used in the transformation of MON531 in MON15985?"
For consistency it was put again in the frame of application GMO/RX-MON15985.

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 2

No comments

Comment 2

Information satisfactory

Comment 3

No questions

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

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

Information satisfactory

Comment 3

No questions

Comment 4

P49
As mentioned by an expert in the evaluation of MON15985 it is indeed a pitty that the second non-functional insert (present in MON531) is not mentioned from the start. However this second non-functional insert is not present in the commercial BC lines and therefore it is of no further importance.

P57
It is stated that MON15985 does not contain "…any detectable plasmid backbone sequence". I would prefer that the authors would add: … backbone sequence from plasmid PV-GHBK11 (since MON15985 does contain plasmid backbone from the plasmid used to construct MON531).

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

Information satisfactory

Comment 3

MON15985 expresses the Cry2 AB_2 and GUS-protein and MON15917 did not affect the levels of Cry1 Ac and NPI II proteins expressed in MON15985 as compared to MON531; this is shown in table 3.1 in seeds and leafs (p. 79 technical dossier).

Levels of Cry-proteins are highest in seeds (important as food and feed are produced from cottonseed) but GUS proteins are half the amount as produced in leafs on fresh weight basis.

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 MON15985 has not been altered in its survival, multiplication and dissemination when compared to conventional cotton varieties. 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.

Comment 2

No comments

Comment 3

Not relevant in the scope of the application for renewal.

Comment 4

No questions

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

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

Segregation analysis shows consistency with a Mendelian inheritance pattern. Additional study on the stability of the first introgressed event MON531 within MON15985 was performed. Demonstration of the stability of the insert has been done. Information and results are sufficient and satisfactory.

Comment 3

No questions

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

Comment 2

No comment

Comment 3

No questions

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 MON15985 was also compared to other commercial conventional cotton varieties. What does it mean? The MON15985 is tolerant to lepidopteran pests. So I think it is not possible to compare with commercial conventional varieties, unless they are also tolerant to lepidopteran pests (= are also genetically modified). My question is : Is MON15985 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:
The same comment has been placed on the EFSAnet for application 42 and a shorter one for application 41.
Final text placed on the EFSAnet:
In this chapter it is mentioned that MON15985 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

Phytic acid has not been determined. Why not?

Comment 3

Compositional analysis reported in Hamilton et al. (2004), from various field trials demonstrated that BollgardII® is comparable in composition to that of the control and conventional cultivar. No significant difference in anti-nutrients was observed.

Comment 4

As for comparative assessment, MON15985 cottonseed composition was compared with non-GM control cottonseed with comparable genetic background as well as with a number of commercial reference varieties; also with literature reported ranges for other varieties.
Although differences were found between MON15985 and control cottonseed for myristic acid, linolenic and arachidic fatty acids, and also for copper, iron and phosphorus content, the range of values were within the 99 % tolerance interval for the commercial varieties. They were also within the reported ranges in literature for other commercial cotton.
The latter range values of course contain two sources of variation: biologically, the differences in cotton varieties compared, and methodologically, the differences in methods, labs, etc.
Overall, the data are convincing that there are no substantial differences in cottonseed or products composition between GM- and non-GM cotton, except for the specific proteins expressed in GM cotton.

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

In this chapter it is mentioned that MON15985 was compared with the control and commercially available cotton varieties, tested in 1998 and 1999. Were those varieties also genetically modified varieties?

Remark SBB:
The same comment has been placed on the EFSAnet for application 42.

Comment 2

Information and experimental set-up are satisfactory.

Comment 3

See 7.1

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

The GUS protein used in this evaluation was over-produced and purified from Escherichia coli (p. 122 of Technical dossier). With regard to microbial protein, Freese and Schubert (2004) mentioned that testing bacterial surrogate proteins should not substitute for testing the plant-expressed proteins.

Remark SBB:
In its guidance document (The EFSA Journal 2004 – 99, 1-94), EFSA states (on p. 28): "It is essential that the tested protein is equivalent to the newly expressed protein as it is expressed in the GM plant. If, due to the lack of sufficient amount of test materials (e.g. plant proteins), a protein is used which was produced by micro-organisms, the structural, biochemical and functional equivalence of the microbial substitute to the newly expressed plant protein must be demonstrated. For example, comparisons of the molecular weight, the isoelectric point, amino acid sequence, post-translational modification, immunological reactivity and, in the case of enzymes, the enzymatic activity, are needed to provide evidence for the equivalence."
There does not seem to have information in the dossier demonstrating the equivalence.

Comment 2

Information and the choice of the studied parameters are satisfactory. However I would like to stress on the importance of the non target approaches as functional genomics, proteomics, or metabolomics (see Molnar, 2005; Yonekura-Sakakibara and Saito, 2006). Given the recent advances in this field such information should be presented, even partially, in an application renewal.

Remark SBB:
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 3

No questions

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

No comment

Comment 2

No questions

D.7.5 Product specification

Comments/Questions of the expert(s)

Comment 1

No comment

Comment 2

No questions

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1

No comment

Comment 2

No questions

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

No comment

Comment 2

No questions

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

Cry1Ac protein measured in MON 15985.

Tissue	ng/mg Tissue Fresh Weight		Standard deviation
	Mean	Range	
Leaf	2.75	0.39-4.19	1.32
Seed	3.35	2.21-4.84	0.63
Whole plant	0.17	0.10-0.32	0.08
Pollen	0.02	0.01-0.02	0.01

Please provide data based on dry weight.

Cry2Ab2 protein measured in MON 15985.

Tissue	ng/mg Tissue Fresh Weight		Standard deviation
	Mean	Range	
Leaf	23.8	10.1-33.3	6.3
Seed	43.2	31.8-50.7	5.7
Whole plant	8.80	7.28-10.46	1.20
Pollen	< 0.25		

Please provide data based on dry weight.

NPTII protein measured in MON 15985.

Tissue	ng/mg Tissue Fresh Weight		Standard deviation
	Mean	Range	
Leaf	16.6	7.53-33.7	5.2
Seed	10.8	8.88-13.2	1.2

Please provide data based on dry weight.

GUS protein measured in MON 15985.

Tissue	ng/mg Tissue Fresh Weight		Standard deviation
	Mean	Range	
Leaf	106	51.7-176	32
Seed	58.8	37.2-82.3	13.0

Please provide data based on dry weight.

Comment 2

Hamilton et al. (2004) reported that Bollgard II, this is the trade name for MON15985 cotton, is as safe as conventional cotton for food and feed use.

Comment 3

Information provided is sufficient.

Comment 4

The functionality of the Cry-proteins as delta-endotoxins is well explained.
It has been shown on mammalian cells that mammals are not susceptible to Cry-proteins including Cry2 Ab, since no receptors for Cry proteins have been identified on intestinal cells (rats, rabbits).
Subchronic and chronic feeding studies as well as acute oral, dermal and inhalation studies have shown the absence of mammalian toxicity for Cry proteins.
The safety studies for Cry2 AB2 and Cry2 Aa protein (for which there is a 97 % homology between the Cry from Bacillus thuringiensis and the Cry expressed in MON15985) include:

- in vivo digestive fate: half life less than 15 seconds in simulated gastric or intestinal fluid
- no homology to known protein allergens
- no homology to known protein toxins
- no effect at highest dose tested (1450 mg/kg)

The latter amount of Cry2 Ab_2 protein given to mouse (in mouse gavage study) is 6.3 x 10^3 higher than the highest dose a cow normally would consume when given whole cottonseed ("worst case scenario").
As for the GUS protein, a β-D-glucuronidase that catalyses the hydrolysis of a range of β-glucuronides into their corresponding acid and aglycones, it is important to know that additional GUS besides the GUS activity already present in gut epithelium, microflora and foods, has no detrimental effect.
GUS activity is indeed important in the efficiency of the entero-hepatic cycle of a number of secreted products from the liver under glucuronide form.
Digestion of GUS protein in simulated gastric and intestinal fluids (99 % of plant GUS digested within 15 seconds) no similarity with toxins, no adverse effects with mouse gavage studies with a safety margin of the highest dose being 2.2 x 10^2 higher than the highest dose a cow normally would consume when given whole cottonseed, allow the conclusion of safety of the newly expressed proteins in MON15985.

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

Degradation of the Cry1Ac protein in simulated gastric fluid ().
Has already been tested previously. No further testing is needed.

Degradation of the Cry1Ac protein in simulated intestinal fluid ().
Has already been tested previously. No further testing is needed.

Cry1Ac: Acute Oral Toxicity Study in Mice ().
Has already been tested previously. No further testing is needed.

Degradation of the NPTII protein in simulated gastric fluid ().
Has already been tested previously. No further testing is needed.

Degradation of the NPTII protein in simulated intestinal fluid ().
Has already been tested previously. No further testing is needed.

NPTII: Acute Oral Toxicity Study in Mice ().
Has already been tested previously. No further testing is needed.

Degradation of the Cry2Ab2 protein in simulated gastric fluid (Leach *et al.*, 2000).
More than 98% of the Cry2Ab2 protein was digested within 15 seconds and that no fragments ≥2 kDa of the parent protein were resolved.

Degradation of the Cry2Ab2 protein in simulated intestinal fluid (Leach *et al.*, 2000).
Immunoblot analysis of SIF incubations showed that a relatively stable Cry2Ab2 protein fragment (~50 kDa) was produced within 1 minute and observed for at least 24 hours. Cry1, Cry2 and Cry3 class proteins yield stable, tryptic core fragments when incubated in SIF (Monsanto, 1997).

Cry2Ab2: Acute Oral Toxicity Study in Mice (Bechtel, 2000).
Three groups of ten male and ten female mice were given acute, oral dosages of Cry2Ab2 protein at 67.3, 359 or 1450 mg/kg body weight, respectively. A separate group of ten male and ten female protein control animals received bovine serum albumin at a dose of 1200 mg/kg. The doses administered were designed to evaluate the potential hazards of the Cry2Ab2 protein at the highest acute oral dose that could be delivered to mice.
There were no adverse effects attributed to the oral administration of Cry2Ab2 protein in male and female mice at doses of 67.3, 359, or 1450 mg/kg body weight. The NOEL for toxicity of Cry2Ab2 protein administered as an acute dose by gavage to mice was considered to be at least 1450 mg/kg, the highest tested dose.

Sequence homology with known toxins (Hileman and Astwood, 2000).
A database of 4677 protein sequences associated with toxicity has been assembled from publicly available genetic databases (GenBank, EMBL, PIR and SwissProt).

The deduced amino acid sequence of the Cry2Ab2 protein was compared to sequences in this toxin database using the FASTA sequence alignment tool.
Not homologous to known protein toxins or other proteins of concern to human health.

Degradation of the GUS protein in simulated gastric fluid (Bonner *et al.*, 2003b).
The results of the SGF study demonstrated that the full length protein was digested within 15 seconds and at least 99% of the plant produced GUS protein, including proteolytic fragments, was digested within four minutes. The SGF results further demonstrated that GUS enzymatic activity was lost within 15 seconds of incubation of SGF.

Degradation of the GUS protein in simulated intestinal fluid (Bonner *et al.*, 2003b).
The results of the SIF study demonstrated that at least 98% of the full length GUS protein was not detectable after six hours of incubation in SIF.

GUS: Acute Oral Toxicity Study in Mice (Naylor, 1992).
There were no treatment-related adverse effects in mice administered GUS protein by oral gavage at actual dosages up to 69 mg/kg, the highest dose tested. There were no statistically significant differences in body weight, cumulative body weight or food consumption between the vehicle or bovine serum albumin protein control groups and GUS protein-treated groups.

Both Cry2Ab2 and GUS are present in cottonseed at approximately the same level. The highest tested dose for Cry2Ab2 is 1450 mg/kg, for GUS it is 69 mg/kg. What is the reason for this major difference?

Sequence homology with known toxins (McCoy *et al.*, 2002a).
A database of protein sequences associated with toxicity was assembled from publicly available genetic databases (GenBank, EMBL, PIR and SwissProt).
The amino acid sequence of the GUS protein was compared to protein sequences in the toxin database using the FASTA sequence alignment tool.
The test sequence, GUS E377K, shared sequence similarities to homologous GUS proteins, as expected. No other significant structural homology was observed.

Comment 2

Information is satisfactory.

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Comment 1

No comment

Comment 2

No questions

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1

In agreement with the provided information.

Comment 2

No questions

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

42-day poultry feeding study (author)

Not performed. No further testing is needed.

Remark SBB:
In applications 41 and 42, the following comment has been placed on the EFSAnet:
"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. 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."
For consistency it was put again in the frame of application GMO/RX-MON15985.

90-Day rat feeding study (Lemen, 2004).

This study compared different parameters in Crl:CD®(SD)IGS BR rats fed a diet containing ground cottonseed from line DP50BX containing event 15985 (2% and 5% w/w in the diet) to (1) a diet containing ground cottonseed from its non-transgenic control line (DP50; 2% and 5% w/w in the diet) and (2) a population of diets containing ground cottonseed from commercial non-transgenic cotton varieties (Chaco 520, Guazuncho, Pora, DP5415, DP5690, and ST474; all reference controls, 5% w/w in the diet) for at least 13 weeks. Twenty rats/sex were assigned to each group.

There was no test material-related **mortality** or **moribundity**. Two rats died in the control groups. No deaths occurred in the test groups.

No test-material-related differences in **body weight** or cumulative body weight changes were observed.

No test-material-related changes in **food consumption** were observed.

There was no evidence in the **clinical pathology** data of any test material-related effects.

No changes attributable to dietary administration of ground cottonseed from line DP50XB containing event 15985 were observed in **organ weights**, **macroscopic findings**, or **microscopic findings**.

No further testing is needed.

Comment 2

At this stage there is no evidence for newly expressed proteins toxicity.

Comment 3

The safety of the respective proteins within MON15985 was further confirmed by repeat-dose animal feeding studies in rats and in catfish using ground cottonseed.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

Cry2Ab2 shares no structurally-significant sequence similarity to sequences within the allergen database and does not share potential immunologically-relevant amino acid sequences greater than seven contiguous identical amino acids. It is not similar to any toxin or other protein relevant to animal or human health. Cry2Ab2 protein was digested in simulated gastric and intestinal fluids (P.115, Technical dossier). However, Bannon et al. (2003) and Herman et al. (2006) concluded that the use of the SGF technique to predict the allergenic status of the proteins remains uncertain. This in vitro assessment of Cry2Ab2 protein digestibility indicates that the Cry2Ab2 protein will be readily digested in the mammalian digestive tract. Nevertheless, Spök et al (2005) have shown that digestibility studies can not be considered as suitable tools to address the allergenic potential of a protein. Although resistance to proteolysis is a characteristic of some allergens, Meredith (2005) stated that there are also many proteins that are known allergens that are susceptible to proteolysis.

Comment 2

Assessment of the allergenicity of the newly expressed proteins.
The reviewer agrees that the newly introduced proteins are not likely to represent allergy risk.

If Cry1Ac is not likely to be an allergen itself, it should be emphasized that Cry1Ac has been proposed as an adjuvant for vaccines (1, 2, 3, 4), 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, 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 MON15985 cottonseeds.

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

The applicant did not evaluate the potential allergenicity of MON15985 cottonseeds, compared to their traditional counterpart. The reviewer agrees, however, that cottonseed allergy is not a major issue. Furthermore, no major allergen of cottonseed has been defined. However, because the introduction of the new traits might influence the expression levels of other proteins of the host plant, it might be relevant to evaluate the content of 2S storage protein and of vicillin, two known common and potent seed allergens, in the MON15985 cottonseed.

Remark SBB:
The same kind of comment has been placed on the EFSAnet for applications 41, 42 and 51.

Comment 3

None of the newly expressed proteins in MON15985 has allergenic potential based on characteristics of known allergenic proteins such as allergenic source of gene, stability to digestion, similarity sequence to allergens, prevalant protein in food. The Cry2 Ab_2 and GUS proteins do not show any of these characteristics.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1

It is noted that the introduced trait is of agronomic interest and is not intended to change any nutritional aspects of this cotton. Can this be proved by data?

Comment 2

Several cottonseed components differed ($P<0.05$) between the control and MON15984 cotton: myristic acid, stearic acid, linolenic acid, arachidonic acid, dihydrosterculic acid, copper, iron, phosphorus, but nevertheless, they were all within the commercial range.
Part of the dossier does not deal with MON15984 cotton, but with MON15984x1445 cotton: Appendix II, III and IV.

Comment 3

No comment

Comment 4

As catfish in commercial systems is consuming large amount of cottonseed meal as a feed ingredient (up to 20 %) this species was used for the nutritional assessment of the GM-cotton.
No differences were found in growth, feed conversion or body composition of the fish fed GM or non-GM cottonseed meal and therefore nutritional equivalency was proven.

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

Comments/Questions of the expert(s)

Comment 1

No comment

Comment 2

No comment

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

Comments/Questions of the expert(s)

Comment 1

No comment

Comment 2

Not applicable

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

D.9.1. Persistence and invasiveness

Comments/Questions of the expert(s)

Comment 1

Not applicable

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

Comment 1

Not applicable

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

Comment 1

Not applicable

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

Comment 1

Not applicable

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

Comments/Questions of the expert(s)

Comment 1

Not applicable

D.9.6 Effects on human health

Comments/Questions of the expert(s)

Comment 1

Because cottonseed oil is mostly used for human food, in contradiction with cottonseed meal, which is mostly used as animal feed, it is unlikely that there is any significant dietary exposure to any cotton protein.

Comment 2

Not applicable

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1

MON15985 is compositionally equivalent to conventional cotton.
The antinutrients cyclopropenoid fatty acids and gossypol in MON15985 cotton are within the nontransgenic reference range.
Feed administration by gavage to mice and the results of a 13-week subchronic feeding study with rats where diets were fed containing ground MON15985 cottonseed at concentrations of up to 5% w/w, and the feeding of MON15985 cottonseed meal at levels of up to 20% in channel catfish diets, showed that there were no adverse effects.

Comment 2

Not applicable

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

Comment 1

Not applicable

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

Comments/Questions of the expert(s)

Comment 1

Not applicable

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

Comment 1

Not applicable

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

Comment 1

Not applicable

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

Comment 1

Not applicable

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

Comment 1

Not applicable

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

Comments/Questions of the expert(s)

Comment 1

Not applicable

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

Comment 1

Not applicable

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20-11-2008

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Compilation of comments of experts in charge of evaluating the application EFSA/GMO/UK/2008/57
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 17 September 2008
Coordinator: Prof. Philippe Baret
Experts: Jean-Luc Hofs (FUSAGx), Johan Van Waes (ILVO)
Domains of expertise of experts involved: agronomy, breeding, improvement of plants, ecology, plant-insect relations, biodiversity, physiology, insect resistance, biosafety research, cotton
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

INTRODUCTION

Dossier **EFSA/GMO/UK/2008/57** concerns an application of the company **Monsanto** for the renewal of the marketing authorisation of the genetically modified **cotton MON15985** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 20 August 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 environmental aspects[1]. 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

[1] The molecular, allergenicity and toxicity aspects have already been evaluated when assessing dossier EFSA/GMO/RX-MON15985

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).
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

GENERAL COMMENTS

Comments/Questions of the expert(s)

Comment 1

As in a former dossier (f.e. EFSA/GMO/RX-MON1445) I give here some general comments.
According to the dossier the scope of application does not include the authorization for the cultivation of MON15985 cotton seed products in the EU. I refer to some remarks in the dossier EFSA/GMO/RX-MON1445 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. As an agronomical expert I have given some comments in the questionnaire of the dossier EFSA/GMO/RX-MON1445, related to cultivation and the environmental aspect, and which can also be relevant in this dossier.

Comment 2 ***(SBB)***

SBB suggests to forward the following comment to EFSA (taken mainly from the letter that the Council sent recently to EFSA):

"This application relates to the same GMO as application EFSA/GMO/RX-MON15985. Therefore, comments made by the Biosafety Advisory Council for the latter application are also valid for the current application. Given the fact that the scope of the current application also covers import and processing, some additional comments relating to environmental and monitoring aspects are presented below.

The Biosafety Advisory Council would also like to draw the attention of EFSA on difficulties it has encountered in the frame of the evaluation of separate applications related to the same GMO. The evaluation of such similar applications would be greatly facilitated if the information provided in the different dossiers could be easily compared. This is however only possible if the presentation of the information follows the same structure and if new or additional information in one application as compared to the other is clearly highlighted. This is obviously not the case for applications GMO/RX-MON15985 and EFSA/GMO/UK/2008/57.

The Biosafety Council would therefore appreciate if EFSA could ensure that in different applications related to the same GMO:

- *Data are organised and presented in the same way*
- *Any new information in comparison with a previous application for the same GMO is clearly identified as such (as it is for example the case for the recently available application GMO/UK/2008/60)*
- *Data provided correspond to the latest available scientific information (in particular in the case of application for renewal of an existing authorisation)"*

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

none

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

ALREADY ASSESSED IN APPLICATION EFSA/GMO/RX-MON15985

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

ALREADY ASSESSED IN APPLICATION EFSA/GMO/RX-MON15985

D. INFORMATION RELATING TO THE GM PLANT

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

ALREADY ASSESSED IN APPLICATION EFSA/GMO/RX-MON15985

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

ALREADY ASSESSED IN APPLICATION EFSA/GMO/RX-MON15985

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

ALREADY ASSESSED IN APPLICATION EFSA/GMO/RX-MON15985

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

ALREADY ASSESSED IN APPLICATION EFSA/GMO/RX-MON15985

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

ALREADY ASSESSED IN APPLICATION EFSA/GMO/RX-MON15985

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

ALREADY ASSESSED IN APPLICATION EFSA/GMO/RX-MON15985

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

ALREADY ASSESSED IN APPLICATION EFSA/GMO/RX-MON15985

Additional comment from Coordinator

"There will be negligible human or animal dietary exposure to the Cry2Ab2 protein present in MON 15985. The human consumable fractions of cotton are cottonseed oil and food additives produced from linters (Jones and King, 1993). These products are processed both chemically and thermally such that all proteins, including the Bacillus thuringiensis protein, would be removed or denatured (Sims and Berberich, 1996; Sims et al., 1996)" (Technical file p. 102).

It is confusing to cluster human and animal use. Grains are used in animal diet and they are far less processed than oil. If cotton seed is used in animal production, a specific discussion on cottonseed toxicity should be provided. In absence of such a discussion, authorisation should be restricted to cotton oil use.

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

Comments/Questions of the expert(s)

Comment 1

No comment

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

D.9.1. Persistence and invasiveness

Comments/Questions of the expert(s)

Comment 1

In this chapter it is mentioned that “Like for conventional cotton, the likelihood of MON15985 spreading in the environment is negligible.? This sentence is imprecise : what is the meaning of “spreading” ? can this phenomenon be quantified ?

Comment 2

Feral GM *G. hirsutum* populations may survive over several years but there is no obvious evidence of invasiveness. In mild and dry winter conditions, the existence of feral perennial populations of *G. hirsutum* (RR and Bt cultivars) along roadsides is highly probable (Hofs *et al*., 2006; Hofs *et al*., 2007). Their persistence depends on 1) public sector: the national or regional infrastructure maintenance policy; which is highly variable in Southern Europe. 2) private sector: facility maintenance of the seed importer, but what are the tangible evidences of the control (frequency, strictness…)?
Occasional non-GM feral plants of *G. hirsutum* have already been reported in Southern Europe and in the Balkans (Flora Europea, 2005; Polumin, 2005).

Cotton doesn't need an arable surface to grow. In the case of seed spillage some seeds can germinate on the top of decomposing seeds, which act as a growth substrate (see picture in annexe). When the cotton root system developing in that cotton compost is strong enough it can pass through a harder surface (road coating).

Remark SBB:

The same kind of comment has been placed on the EFSAnet for other applications concerning import and processing of GM cotton.

Additional comment from the coordinator

D 9.1. : « Based on centuries of experience with traditional cotton in the EU, there is negligible potential for cotton to be invasive of natural habitats or to persist in the agronomic environment without the aid of human intervention. This is because cotton is a poor competitor, and outside of cultivation has no meaningful impact on the environment. » This assertion is not proven : no references, no precise data. What is meant by negligible ? Does is mean « a low occurrence » or «without consequences » ?

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

Comment 1

Negligible risks: see remark as in D 9.1: can this be quantified?

Comment 2

Cry1Ac toxin controls Noctuidae Lepidoptera, as *Helicoverpa (Heliothis) armigera*. This species covers the southern part of Europe (South of France, Spain, Italy, Greece…) (Bues et al,1989). Cry2Ab controls partially *Spodoptera sp*. (see the review of Sanchis and Bourget, 2008) and *S. exigua* is present in France (Bues,2006) and other Mediterranean countries. Cry toxins expressed in the GM plants give a selective advantage when these insect species are present in the environment. The selective advantage of an allele is the most important factor before dispersion rate, mitigation rate and plant fecundity /multiplication rate (Chapman and Burke, 2006).
This trait can thus contribute in reducing potential causes of feral population (partial) control by bollworms. The extent of such risk should be investigated in depth before authorization.

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

Comment 1

Negligible likelihood for gene transfer: see remark as in D 9.1: can this be quantified?

Comment 2

If feral plants are more than 25m apart from a cultivated conventional cotton field, the risk of gene transfer is negligible (references in the application should be completed with Van Deynze et al., 2005; Llywellyn et al., 2007). In some cases road side populations from seed spillage could be a problem in a cotton production area.

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

Comment 1

As feral cotton is supposed not to spread in a large extent, interactions between the GM plant and target organisms are negligible.

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

Comments/Questions of the expert(s)

Comment 1

Negligible risk for harmful effects: : see remark as in D 9.1: can this be quantified?

Comment 2

As feral cotton are suppose not to spread in a large extent, interactions between the GM plant and non target organisms are negligible.

D.9.6 Effects on human health

Comments/Questions of the expert(s)

Comment 1

No comment

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1

No comment

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

Comment 1

Information is satisfactory.

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

Comments/Questions of the expert(s)

Comment 1

Not applicable.

Comment 2

No comment

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

Comment 1

No comment

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

Comment 1

Not applicable since no demand for cultivation.

Comment 2

Who controls effectively the strict application of the monitoring plan?
Road maintenance services are not mentioned as primary source of information or “detection” of seed/plant escape. Should they be part of the General Surveillance Plan?

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

Comment 1

Not applicable

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

Comment 1

Not applicable

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

Comments/Questions of the expert(s)

Comment 1

Not applicable

Comment 2

See D.11.1.

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

Comment 1

Not applicable

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