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Scientific opinion on application (EFSA-GMO-NL-2011-96) for the placing on the market of genetically modified insect-resistant and herbicide-tolerant cotton GHB119, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience AG

EFSA Panel on Genetically Modified Organisms (GMO)

Abstract

Cotton GHB119 was developed by *Agrobacterium tumefaciens*-mediated transformation. It expresses the Cry2Ae and phosphinothricin acetyltransferase (PAT) proteins which, respectively, confer resistance to certain lepidopteran species and tolerance to glufosinate ammonium-based herbicides. The molecular characterisation of cotton GHB119 did not give rise to safety issues. The agronomic, phenotypic and compositional characteristics of cotton GHB119 tested under field conditions revealed no relevant differences between cotton GHB119 and its conventional counterpart that would give rise to any food and feed or environmental safety concern. There were no concerns regarding the potential toxicity and allergenicity of the newly expressed proteins Cry2Ae and PAT, and no evidence that the genetic modification might significantly change the overall allergenicity of cotton GHB119. The nutritional characteristics of cotton GHB119 are not expected to differ from those of non-GM cotton varieties. There are no indications of an increased likelihood of establishment and spread of feral cotton GHB119 plants. Considering the scope of this application, interactions with the biotic and abiotic environment were not considered to be an issue. Risks associated with an unlikely but theoretically possible horizontal gene transfer from cotton GHB119 to bacteria have not been identified. The monitoring plan and reporting intervals are in line with the scope of the application. In conclusion, the EFSA GMO Panel considers that the information available for cotton GHB119 addresses the scientific comments raised by the Member States and that cotton GHB119, as described in this application, is as safe as its conventional counterpart with respect to potential effects on human and animal health and the environment in the context of the scope of this application.

Keywords: GMO, cotton GHB119, Regulation (EC) No 1829/2003, Cry2Ae, PAT, insect resistance, herbicide tolerance, import and processing

Requestor: Competent Authority of the Netherlands

Question number: EFSA-Q-2011-00311

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Summary

Following the submission of an application (EFSA-GMO-NL-2011-96) under Regulation (EC) No 1829/2003 from Bayer CropScience AG, the Panel on Genetically Modified Organisms of the European Food Safety Authority (GMO Panel) was asked to deliver a scientific opinion on the safety of insect-resistant and herbicide-tolerant genetically modified (GM) cotton GHB119 (Unique Identifier BCS-GHØØ5-8). The scope of the application EFSA-GMO-NL-2011-96 is for import, processing, and food and feed uses of cotton GHB119 within the European Union (EU), but excludes cultivation in the EU.

The GMO Panel evaluated cotton GHB119 with reference to the scope and appropriate principles described in its guidelines for the risk assessment of GM plants. The evaluation addressed the following components of the risk assessment: the molecular characterisation of the inserted DNA and analysis of the expression of the corresponding proteins; the comparative analyses of compositional, agronomic and phenotypic characteristics; the safety of the newly expressed proteins and the whole food/feed with respect to potential toxicity, allergenicity and nutritional characteristics; and the environmental risk assessment and the post-market environmental monitoring (PMEM) plan.

Cotton GHB119 was developed by *Agrobacterium tumefaciens*-mediated transformation. It expresses the Cry2Ae and phosphinothricin acetyltransferase (PAT) proteins which, respectively, confer resistance to certain lepidopteran species, and tolerance to glufosinate ammonium-based herbicides. The molecular characterisation data establish that cotton GHB119 contains a single insert consisting of a single copy of the T-DNA containing the *cry2Ae* and the *bar* expression cassettes. No other parts of the plasmid used for transformation are present in cotton GHB119. Bioinformatic analyses and genetic stability studies were performed and the results did not give rise to safety issues. The levels of the newly expressed proteins present in cotton GHB119 were obtained and reported adequately.

The agronomic, phenotypic and compositional characteristics of cotton GHB119 were compared with those of the conventional counterpart Coker 312 under field conditions. Differences were noted in cotton GHB119 for several agronomic endpoints, but they did not give rise to any food and feed or environmental safety concern. No differences requiring further assessment with regard to safety by the GMO Panel were identified at analyses of compositional data of fuzzy cottonseeds from cotton GHB119, except for the decreased levels of crude protein and tocopherols. As crude protein levels in cotton GHB119 in some trials fell outside the range described in the OECD consensus document (OECD, 2009), and as cottonseed is a relevant source of vegetable oil due to its high tocopherol content, further food and feed nutritional assessment was performed and no safety concerns were identified. The nutritional characteristics of food and feed derived from cotton GHB119 are not expected to differ from that of food and feed derived from non-GM cotton varieties. The safety assessment identified no concerns regarding the potential toxicity or allergenicity of the newly expressed Cry2Ae and PAT proteins in cotton GHB119, and found no indications of a potentially increased overall allergenicity of cotton GHB119 with respect to non-GM cotton. The GMO Panel concludes that cotton GHB119 is as safe and nutritious as its conventional counterpart. The GMO Panel considers that post-market monitoring of food/feed derived from cotton GHB119 is not necessary, given the absence of safety concerns identified.

Application EFSA-GMO-NL-2011-96 covers the import, processing, and food and feed uses of cotton GHB119, and excludes cultivation. Therefore, there is no requirement for a scientific assessment of possible environmental effects associated with the cultivation of this GM cotton. The GMO Panel concluded that there are no indications of an increased likelihood of establishment and spread of feral cotton GHB119 plants in case of accidental release into the environment of viable GM cotton seeds. Potential interactions with the biotic and abiotic environment were not considered to be an issue by the GMO Panel. Risks associated with an unlikely but theoretically possible horizontal gene transfer from cotton GHB119 to bacteria have not been identified. The PMEM plan provided by the applicant is in line with the scope of the application and the requirements of the GMO Panel for PMEM of GM plants. The GMO Panel agrees with the reporting intervals proposed by the applicant in the monitoring plan.

In delivering its scientific opinion, the GMO Panel took into account the information presented in application EFSA-GMO-NL-2011-96, additional information provided by the applicant, scientific

comments submitted by the Member States and relevant scientific publications. In conclusion, the GMO Panel considers that the information available for cotton GHB119 addresses the scientific comments raised by the Member States and that cotton GHB119, as described in this application, is as safe as its conventional counterpart with respect to potential effects on human and animal health and the environment in the context of the scope of this application.

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1. Introduction

Cotton GHB119 was developed to confer resistance to certain lepidopteran pests and tolerance to glufosinate ammonium-based herbicides. Resistance to pests, such as the cotton bollworm larvae (CBW, *Helicoverpa zea*), tobacco budworm larvae (TBW, *Heliothis virescens*) and fall armyworm larvae (FAW, *Spodoptera frugiperda*), is provided by the expression of the Cry2Ae protein, which has an insecticidal effect on larvae of certain lepidopteran species. Tolerance to glufosinate ammonium-based herbicides is achieved by the expression of phosphinothricin acetyltranferase (PAT), an enzyme that acetylates L-glufosinate ammonium.

1.1. Background

On 7 April 2011, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands an application, EFSA-GMO-NL-2011-96, for authorisation of genetically modified (GM) cotton GHB119 for food and feed uses, import and processing submitted by Bayer CropScience AG within the framework of Regulation (EC) No 1829/2003[1] on GM food and feed.

After receiving the application EFSA-GMO-NL-2011-96 and in accordance with Articles 5(2)(b) and 17(2)(b) of the Regulation (EC) No 1829/2003, EFSA informed the Member States and the European Commission, and made the summary of the application publicly available on the EFSA website. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of the Regulation (EC) No 1829/2003. On 30 August 2011 and 31 October 2011, EFSA received additional information requested under the completeness check (requested on 26 May 2011 and 19 September 2011, respectively). On 21 November 2011, EFSA declared the application valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to the Member States and the European Commission, and consulted nominated risk assessment bodies of the Member States, including the national Competent Authorities within the meaning of Directive 2001/18/EC[2] following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. The Member States had 3 months after the opening of the Member State commenting period (until 21 February 2012) to make their opinion known.

The GMO Panel carried out a scientific risk assessment of GM cotton GHB119. On 14 February 2012, 17 April 2012, 11 February 2013, 22 and 28 February 2013, 24 July 2013, 24 June 2015, 5 October 2015, 12 October 2015, 26 November 2015, 16 February 2016, 7 April 2016, 13 June 2016 and 3 August 2016, the GMO Panel requested additional information from the applicant. The applicant provided the requested information on 21 May 2012, 4 July 2012, 13 March 2013, 5 April 2013, 2 March 2015, 9 July 2015, 11 November 2015, 30 November 2015, 23 December 2015, 4 April 2016, 16 June 2016, 29 June 2016 and 25 August 2016. After receipt and assessment of the full data package, the GMO Panel finalised its risk assessment of cotton GHB119.

In giving its scientific opinion on cotton GHB119 to the European Commission, the Member States and the applicant, and in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003, EFSA has endeavoured to respect a time limit of 6 months from the acknowledgement of the valid application. As additional information was requested by the GMO Panel, the time limit of 6 months was extended accordingly, in line with Articles 6(1), 6(2), 18(1) and 18(2) of Regulation (EC) No 1829/2003.

According to Regulation (EC) No 1829/2003, this scientific opinion is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation and thus will be part of the EFSA overall opinion in accordance with Articles 6(5) and 18(5).

1.2. Terms of Reference as provided by the requestor

The EFSA GMO Panel was requested to carry out a scientific assessment of cotton GHB119 (*Gossypium hirsutum*) for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003.

Where applicable, any conditions or restrictions which should be imposed on the placing on the market and/or specific conditions or restrictions for use and handling, including post-market monitoring (PMM) requirements based on the outcome of the risk assessment and, in the case of GMOs

[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. 1–23.

[2] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 12.3.2001, p. 1–38.

or food/feed containing or consisting of GMOs, conditions for the protection of particular ecosystems/ environments and/or geographical areas should be indicated in accordance with Articles 6(5)(e) and 18(5)(e) of Regulation (EC) No 1829/2003.

The EFSA GMO Panel was not requested to give an opinion on the information required under Annex II to the Cartagena Protocol. Furthermore, the EFSA GMO Panel did not consider proposals for labelling and methods of detection (including sampling and the identification of the specific transformation event in the food/feed and/or food/feed produced from it), which are matters related to risk management.

2. Data and methodologies

2.1. Data

In delivering its scientific opinion, the GMO Panel took into account application EFSA-GMO-NL-2011-96, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications.

2.2. Methodologies

The GMO Panel carried out a scientific risk assessment of cotton GHB119 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. The GMO Panel took into account the appropriate principles described in its guidelines for the risk assessment of genetically modified (GM) plants and derived food and feed (EFSA, 2006a; EFSA GMO Panel, 2011a), the environmental risk assessment of GM plants (EFSA GMO Panel, 2010) and on the post-market environmental monitoring of GM plants (EFSA, 2006b; EFSA GMO Panel, 2011b).

The comments raised by the Member States are addressed in Annex G of EFSA's overall opinion[3] and were taken into consideration during the scientific risk assessment.

3. Assessment

3.1. Molecular characterisation

3.1.1. Evaluation of relevant scientific data

3.1.1.1. Transformation process and vector constructs

Cotton GHB119 was developed by *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*)-mediated transformation. The embryogenic calli of cotton (*G. hirsutum*) variety Coker 312 were cocultured with a disarmed *A. tumefaciens* strain C58C1 containing the vector pTEM12.[4]

The pTEM12 vector includes one T-DNA, which contains two gene cassettes:[5] *cry2Ae* and *bar*.

The *cry2Ae* cassette, which confers insect resistance, contains the following genetic elements: the *Cauliflower Mosaic Virus* 35S promoter (p35S2); the 5′ leader sequence of the chlorophyll a/b binding protein encoding gene from *Petunia hybrida* (5′*cab*22L); the sequence encoding the transit peptide of the ribulose-1,5-bisphosphate carboxylase small subunit from the gene *ats1A* of *Arabidopsis thaliana* (TpssuAt); the codon-optimised *cry2Ae* sequence from *Bacillus thuringiensis* subsp. *dakota* 1715; and the 3′ untranslated region of the 35S transcript of the *Cauliflower Mosaic Virus*.

The *bar* cassette conferring tolerance to glufosinate ammonium contains the following genetic elements: the promoter of *Cassava Vein Mosaic Virus* (Pcsvmv); the *bar* gene from *Streptomyces hygroscopicus* strain ATCC 21705, coding for the PAT protein, with an altered amino acid (from Ser to Asp) in the second codon[6]; and the 3′ untranslated region of the nopaline synthase gene from the T-DNA of pTiT37.

The vector backbone sequence contains the aminoglycoside adenyltransferase (*aadA*) gene conferring bacterial resistance to spectinomycin; a fragment of the neomycin phosphotransferase gene *nptII*; the origin of replication from the *Escherichia coli* plasmid pBR322; the origin of replication from

[3] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2011-00311
[4] Dossier: Part I – Section C1.
[5] Dossier: Part I – Section C2.
[6] Dossier: Part I – Appendices (Report ID M-084188-01-2).

the *Pseudomonas* plasmid pVS1; and the residual plasmid sequences from the *A. tumefaciens* plasmid pTiAch5.

3.1.1.2. Transgene constructs in the genetically modified plant

Molecular characterisation of cotton event GHB119 was performed by Southern analysis and sequencing of polymerase chain reaction (PCR)-amplified DNA fragments to determine copy number, size and organisation of the inserted sequences, and to confirm the absence of plasmid backbone sequence in cotton GHB119.[7] The approach used was acceptable both in terms of coverage and sensitivity.

Southern analysis with a set of eight restriction enzymes used individually or in combination, together with seven distinct probes, indicated that the cotton event GHB119 contains a single insert, which consists of a single copy of the T-DNA with the same configuration as in the pTEM12 transformation vector. The absence of vector backbone was tested by Southern analysis, using six overlapping probes.[8]

The insert and 1,019 bp and 1,026 bp of the 5′ and 3′ flanking regions of cotton GHB119, respectively, were sequenced. The insert of 4,302 bp is identical to the T-DNA of pTEM12. The sequencing of the pre-insertion locus was determined, and the comparison of its sequence to the sequence of the 5′ and 3′ flanking regions of cotton GHB119 showed that a deletion of 8 bp of plant DNA occurred at the insertion site as a result of the transformation.[9] The possible interruption of known endogenous cotton genes by the insertion in GHB119 was evaluated by bioinformatic analyses of the pre-insertion locus and of the genomic sequences flanking the insert.[10] The results from these analyses did not indicate the interruption of any known endogenous gene in cotton GHB119 and, together with results of segregation (see Section 3.1.1.4), established that the insert is located in the nuclear genome.

Updated bioinformatic analyses[11] of the amino acid sequence of the newly expressed Cry2Ae and PAT proteins revealed no significant similarities to toxins or allergens. Using an 80-amino acids sliding window approach, no significant similarity over 35% identity with known allergens was found for the Cry2Ae and PAT proteins. In addition, updated bioinformatic analyses[11] of the newly created open reading frames (ORFs) within the insert and at its junction sites indicate that expression of an ORF showing significant similarities to toxins and allergens is highly unlikely.

3.1.1.3. Information on the expression of the insert

Levels of the Cry2Ae and PAT proteins were analysed by enzyme-linked immunosorbent assay (ELISA) from cottonseeds of cotton GHB119 harvested from six locations in the USA in 2006 and from six locations in Spain in 2007. Samples analysed were obtained from plants treated with glufosinate ammonium-based herbicides or only with conventional herbicides.[12] The mean values and ranges of protein expression levels in cottonseeds of the Cry2Ae and PAT proteins are summarised in Table 1.

Table 1: Mean, standard deviation (upper row) and ranges (lower row) of Cry2Ae and PAT protein levels in cotton GHB119 (μg/g fresh weight)

Field trial year (location)	Cry2Ae		PAT	
	Treated[(a)]	Untreated[(b)]	Treated[(a)]	Untreated[(b)]
2006 (USA)	1.47 ± 0.07	1.55 ± 0.19	49.9 ± 3.5	50.7 ± 4.1
n* = 54	1.38–1.57	1.30–1.81	45.5–56.1	44.5–54.9
2007 (Spain)	3.26 ± 0.45	3.18 ± 0.32	117 ± 25	114 ± 25
n = 36	2.80–3.81	2.75–3.55	96.9–158	86.8–152

PAT: phosphinothricin acetyltransferase.
*n – number of samples tested
(a): Cotton GHB119 treated with glufosinate ammonium-based herbicides.
(b): Cotton GHB119 treated only with conventional herbicides.

[7] Dossier: Part I – Section D2.
[8] Dossier: Part I – Section D2 (a).
[9] Dossier: Part I – Section D2 (c).
[10] Dossier: Part I – Section D2 (b).
[11] Additional information: 16/6/2016.
[12] Dossier: Part I – Section D3 (a), (b) and (d).

3.1.1.4. Inheritance and stability of inserted DNA

Genetic stability of the GHB119 insert was studied by Southern analysis. Samples represented three generations grown at six different field locations. The restriction enzyme/probe combinations used were sufficient to conclude that all the plants tested retained the single copy of the insert and flanking regions, which were stably inherited in multiple generations.[13]

Phenotypic stability was observed by segregation analysis of the herbicide tolerance trait in plants from the F_1 generation of a cross between GHB119/T_0 plants with three conventional genetic backgrounds and from F_2 generation resulting from self-pollination of the F_1 plants. The results supported the presence of a single insertion, segregating in a Mendelian fashion.

3.1.2. Conclusion on molecular characterisation

The molecular characterisation data establish that cotton GHB119 contains a single insert consisting of one copy of the *cry2Ae* and *bar* expression cassettes. Bioinformatic analyses of the sequences encoding the newly expressed proteins and of other ORFs present within the insert or spanning the junction sites between the insert and genomic DNA did not give rise to safety issues. The stability of the inserted DNA and of the herbicide tolerance traits was confirmed over several generations. The levels of the Cry2Ae and PAT proteins were obtained and reported adequately.

3.2. Comparative analysis

3.2.1. Evaluation of relevant scientific data

3.2.1.1. Choice of comparator and production of material for the comparative analysis[14]

Application EFSA-GMO-NL-2011-96 presents data on cottonseed composition of cotton GHB119 derived from field trials performed at six sites in the USA[15] in the 2006 growing season and in Spain during two growing seasons, 2007 and 2008 (Table 2). The Spanish field trials were also used for the agronomic and phenotypic characterisation (Table 2). As indicated in Section 3.1.1.1, GM cotton GHB119 was obtained by transforming cotton variety Coker 312. In these field trials, Coker 312 was used as a comparator. The GMO Panel considers that this non-transgenic line is the appropriate conventional counterpart, as it has the same genetic background as the cotton line of cotton GHB119.

Table 2: Overview of comparative assessment studies with cotton GHB119 and its conventional counterpart Coker 312 provided in application EFSA-GMO-NL-2011-96.

Study details (year, country/region)	Field trial sites		
	Total number of sites	Sites used for agronomic and phenotypic characteristics	Sites used for compositional analysis
2006, USA	6	None	6
2007, Spain (Andalusia)	6	6	6
2007, Spain (Catalonia)	12	12	None
2008, Spain (Andalusia)	6	6	6
2008, Spain (Catalonia)	8	8	None
Aggregate studies in Spain (2007/2008, Andalusia/Catalonia)	32	32	12

The USA 2006 field trials included cotton GHB119 treated with glufosinate ammonium-based herbicides, cotton GHB119 treated only with conventional herbicides and the conventional counterpart (Coker 312) treated with conventional herbicides. In each location, a randomised complete block design with three replications of each material was applied.

[13] Dossier: Part I – Section D5.
[14] Dossier: Part I – Section D7.2.
[15] Arkansas (two sites), Georgia (one site), Mississippi (one site) and Texas (two sites).

In order to investigate additional characteristics of the GM cotton GHB119, pollen morphology and germination were investigated, as well as the overwintering capacity of the cottonseeds of cotton GHB119.

The Spanish field trials were carried out in the regions of Catalonia (12 sites in 2007 and eight in 2008) and Andalusia (six sites in 2007 and six in 2008). In each site, a randomised complete block design was used with three replications of the following materials: cotton GHB119 treated with glufosinate ammonium-based herbicides, cotton GHB119 treated only with conventional herbicides and the conventional counterpart treated with conventional herbicides. Non-GM commercial reference varieties were also included in each location.[16]

Data for agronomic and phenotypic traits were collected from all the 32 field trials in Spain, while the 12 sites from Andalusia (2007/2008) were used for the compositional analysis. Initially, four Catalonian sites (out of 20 in total) were also included in the compositional analysis; however, the selection criteria for those sites were considered not acceptable by the GMO Panel.[17]

3.2.1.2. Agronomic and phenotypic characteristics[18]

The agronomic and phenotypic characteristics collected from the 32 field trial sites in Spain (Table 2) included plant morphology characteristics,[19] parameters related to agronomic performances,[20] interactions with biotic stressors[21] and fibre properties.[22]

The data were analysed with analysis of variance (ANOVA), in order to test for differences between cotton GHB119 and its conventional counterpart Coker 312.[23,24] Statistically significant differences between cotton GHB119 (treated with glufosinate ammonium-based herbicides, 'treated', or treated only with conventional herbicides, 'untreated') and its conventional counterpart (Coker 312) treated with conventional herbicides were identified for all the measured endpoints except for short fibre index (between cotton GHB119 untreated and Coker 312) and for fibre elongation (between cotton GHB119 treated and Coker 312). The potential environmental impact of the observed differences is discussed in Section 3.4.1.1.

The differences in the fibre parameters, such as length, strength and micronaire, did not lead to a different classification of the fibre quality. The differences in endpoints related to insect damage and presence of insect larvae were expected since cotton GHB119 expresses the insecticidal Cry2Ae protein. The potential environmental impact of the observed difference is discussed in Section 3.4.1.1.

In addition, an overwintering study with cotton GHB119 in comparison to Coker 312 was performed in a single location (Lubbock, Texas) in the USA. Seeds produced in 2008 were sampled and subsequently incubated over winter underground. After 7 months, the seeds were inspected and analysed for their viability. The data indicated that cottonseeds derived from cotton GHB119 as well as from Coker 312 do not remain viable after overwintering for 7 months.

Agronomic and phenotypic characteristics tested under controlled conditions[25]

Pollen germinability and viability from cotton GHB119 and its conventional counterpart (Coker 312) were measured according to Barrow (1981). Pollen was obtained from plants grown under greenhouse conditions in 2007. The pollen germinability was tested between 0 and 78 h after its collection. The applicant observed no statistically significant differences between cotton GHB119 and its conventional counterpart for pollen germinability and viability.

While Barrow's test provides an indication of the germination capacity, it does not measure pollen viability directly as germination is forcefully induced. Therefore, the data on pollen viability supplied by the applicant in support of the comparative assessment of cotton GHB119 are not considered suitable

[16] In the Catalonian field trials, two commercial non-GM varieties were included at each location; in the Andalusian field trials, four commercial non-GM varieties were included at each location.
[17] Additional information: 4/4/2016.
[18] Dossier: Part I – Section D4 and D7.4.
[19] Measured plant morphology characteristics: plant height, developed nodes, first bolls position and total number of bolls.
[20] Measured agronomic performances: seed cotton yield, stand count, fibre yield, number of seed/10 bolls, weight fuzzy seed/10 bolls, total bolls, number of days to first flower and number of days to first boll.
[21] Biotic stressors: number of damaged square, flower and boll per 20 plants each at date 1 and 2 produced by insects and by living larvae, and damage by insects at date 2.
[22] Fibre properties: fibre fineness – micronaire, fibre length, fibre length uniformity, short fibre index, fibre strength and fibre elongation.
[23] Additional information: 4/7/2012.
[24] A linear model was used, with the fixed factors regimen (each of the three materials), region (Andalusia/Catalonia), season (2007/2008) and site, and terms for the interaction of regimen with the other factors.
[25] Dossier: Part I – Section D4.

by the GMO Panel. Given that the genetic modification of cotton GHB119 is not designed to target specific pollen and seed characteristics, that cotton is not a persistent and invasive crop, and that the scope of cotton GHB119 excludes cultivation, the GMO Panel considers that data on pollen viability are not essential for the risk assessment of cotton GHB119.

3.2.1.3. Compositional analysis[26]

Fuzzy cottonseeds from the USA field trials in 2006 (Table 2) were analysed for proximates, fibre fractions, amino acids, fatty acids, vitamin E, minerals and anti-nutrients.[27] The choice of the compounds followed the recommendations of the OECD consensus document (OECD, 2009).

The data for compositional analysis from the field trials in the USA in 2006 were analysed with ANOVA,[28] in order to test for differences between cotton GHB119 and its conventional counterpart Coker 312. The statistical analysis identified 24 significant differences between cotton GHB119 (treated with glufosinate ammonium-based herbicides) and its conventional counterpart, and 23 significant differences between cotton GHB119 (treated only with conventional herbicides) and its conventional counterpart (Table 3).

Table 3: Compositional endpoints (USA field trials, 2006) for which significant differences were found between cotton GHB119 and its conventional counterpart Coker 312

Component	cotton GHB119		Conventional counterpart (Coker 312)
	Treated[(a)]	Untreated[(b)]	
Moisture (% FW)	9.99*	10.14	10.57
Crude protein (% DM)	20.01*	20.67*	23.06
Total carbohydrates (% DM)	57.32*	56.26*	54.29
Zinc (mg/kg DM)	36.2*	35.3	34.0
α-tocopherol (mg/kg DM)	118*	121*	128
Alanine (% AA)	4.43*	4.45*	4.35
Arginine (% AA)	11.09*	10.97*	11.59
Glutamic acid (% AA)	20.77*	20.66*	21.01
Glycine (% AA)	4.64*	4.65*	4.58
Histidine (% AA)	3.08*	3.08*	3.04
Leucine (% AA)	6.54*	6.55*	6.44
Lysine (% AA)	5.19*	5.22*	5.05
Proline (% AA)	4.11	4.17*	4.08
Serine (% AA)	5.06*	5.08*	4.87
Threonine (% AA)	3.63*	3.66*	3.51
Tyrosine (% AA)	2.84*	2.85*	2.74
Myristic acid (C14:0) (% FA)	0.82*	0.81*	0.76
Stearic acid (C16:0) (% FA)	2.47*	2.47*	2.36
Oleic acid (C18:1) (% FA)	18.41*	18.28*	15.68
Linoleic acid (C18:2) (% FA)	52.53*	52.67*	55.53
Arachidic acid (C20:0) (% FA)	0.29*	0.30*	0.28
Free gossypol (% DM)	0.530*	0.538*	0.642
Total gossypol (% DM)	0.618*	0.613*	0.714

[26] Dossier: Part I – Section D7.3; additional information: 4/7/2012, 2/3/2015, 9/7/2015 and 4/4/2016.

[27] Proximates and fibre compounds included moisture, crude protein, crude fat, ash, total carbohydrates, acid detergent fibre (ADF) and neutral detergent fibre (NDF); minerals included calcium, phosphorus, potassium, magnesium, iron and zinc; vitamin E compounds included α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol and total tocopherols; amino acids included alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine; fatty acids included myristic (C14:0), palmitic (C16:0), margaric (C17:0), stearic (C18:0), arachidic (C20:0), behenic (C22:0), lignoceric (C24:0), palmitoleic (C16:1), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3); antinutrients included free and total gossypol, phytic acid and the cyclopropenoid fatty acids sterculic, malvalic and dihydrosterculic.

[28] A linear model was used, with the fixed factors regimen (each of the three materials) and site, and terms for the interaction of regimen with the other factors.

Component	cotton GHB119		Conventional counterpart (Coker 312)
	Treated[(a)]	Untreated[(b)]	
Dihydrosterculic acid (% FA)	0.193*	0.198*	0.15
Phytic acid (% DM)	1.38*	1.39*	1.44

FW: fresh weight; DM: dry matter; FA: total fatty acids; AA: total amino acid.
The values shown are estimated means. Significantly different entries for the GM cotton are marked with an asterisk.
The EFSA GMO Panel noted that the free gossypol content in raw cottonseeds of cotton GHB119 and its conventional counterpart was higher than the limits set in Directive 2002/32 EC (5,000 mg/kg as fed) on undesirable substances in feed materials.
(a): Cotton GHB119 treated with glufosinate ammonium-based herbicides.
(b): Cotton GHB119 treated only with conventional herbicides.

Fuzzy cottonseeds from the field trials carried out in Andalusia in 2007 and 2008 (Table 2) were analysed for proximates and fibre, minerals, amino acids, fatty acids, vitamin E and antinutrients.[29] The choice of the compounds followed the recommendations of the OECD consensus document (OECD, 2009).

The data were analysed with ANOVA, in order to test for differences between cotton GHB119 and its conventional counterpart Coker 312.[17,30] The statistical analysis identified 29 significant differences between cotton GHB119 (treated with glufosinate ammonium-based herbicides) and its conventional counterpart, and 32 significant differences between cotton GHB119 (treated only with conventional herbicides) and its conventional counterpart (Table 4).

Table 4: Compositional endpoints (field trials in Andalusia, Spain, 2007/2008) for which significant differences were found between cotton GHB119 and its conventional counterpart Coker 312.

Component	cotton GHB119		Conventional counterpart (Coker 312)
	Treated[(a)]	Untreated[(b)]	
Moisture (% FW)	9.23*	9.11	8.95
Crude protein (% DM)	23.5*	23.5*	27.8
Crude fat (% DM)	23.4*	22.9*	21.5
Ash (% DM)	3.98*	3.92*	4.11
ADF (% DM)	39.8*	39.8*	38.7
NDF (% DM)	48.9*	49.7*	46.5
Total carbohydrate (% DM)	49.1*	49.6*	46.5
Phosphorous (% DM)	0.56	0.53*	0.56
Magnesium (% DM)	0.369*	0.380*	0.445
Iron (mg/kg DM)	45.2*	45.7*	52.5
Zinc (mg/kg DM)	33.7*	35.8*	40.2
Total tocopherol (α-toc. equiv.) (mg/kg DM)	101.7*	107.0*	114.0
Alanine (% AA)	4.18*	4.13*	4.00
Arginine (% AA)	11.55*	11.57*	12.42
Leucine (% AA)	6.29*	6.26*	6.16
Lysine (% AA)	5.07*	5.02*	4.92
Serine (% AA)	3.65	3.72*	3.58

[29] Proximates and fibre compounds included moisture, crude protein, crude fat, ash, total carbohydrates, acid detergent fibre (ADF) and neutral detergent fibre (NDF); minerals included calcium, phosphorus, potassium, magnesium, iron and zinc; anti-nutrients included free gossypol, total gossypol, phytic acid, malvalic acid, sterculic acid and dihydrosterculic acid; vitamin E compounds included total tocopherol; amino acids included alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine; fatty acids included myristic (C14:0), pentadecanoic (C15:0), palmitic (C16:0), palmitoleic (C16:1, ω-7), palmitoleic (C16:1, ω-9), heptadecanoic (C17:0), stearic (C18:0), oleic (C18:1) *cis* isomer, oleic (C18:1), linoleic (C18:2), linoleic (C18:2) *trans* isomer, α-linolenic (C18:3), arachidic (C20:0), gadoleic (C20:1), behenic (C22:0) and lignoceric (C24:0).

[30] A linear model was used, with the fixed factors regimen (each of the three materials), season (2007/2008) and site, and terms for the interaction of regimen with the other factors.

Component	cotton GHB119		Conventional counterpart (Coker 312)
	Treated[(a)]	Untreated[(b)]	
Threonine (% AA)	3.14*	3.14*	3.03
Tryptophan (% AA)	1.46	1.51*	1.41
Tyrosine (% AA)	2.58*	2.59*	2.39
Myristic acid (C14:0) (% FA)	0.89*	0.88*	0.85
Palmitic acid (C16:0) (% FA)	23.05*	23.20	23.38
Palmitoleic acid (C16:1, ω-7) (% FA)	0.64*	0.63*	0.59
Oleic acid (C18:1) (% FA)	19.92*	20.07*	17.75
Oleic acid (C18:1) (*cis*-isomer) (% FA)	0.97*	0.96*	1.02
Linoleic acid (C18:2) (% FA)	49.38*	49.27*	51.64
Linoleic acid (C18:2) (*trans*-isomer) (% FA)	0.046	0.043*	0.046
α-linolenic acid (C18:3) (% FA)	0.160	0.152*	0.162
Arachidic acid (C20:0) (% FA)	0.328*	0.318*	0.304
Behenic acid (C22:0) (% FA)	0.125*	0.116*	0.106
Lignoceric acid (C24:0) (% FA)	0.075	0.072*	0.078
Total gossypol (% DM)	0.75*	0.71	0.71
Malvalic acid (% FA)	0.48*	0.46*	0.43
Sterculic acid (% FA)	0.25*	0.25*	0.19
Dihydrosterculic acid (% FA)	0.27*	0.26*	0.19

FW: fresh weight; DM: dry matter; ADF: acid detergent fibre; NDF: neutral detergent fibre; AA: total amino acid; FA: total fatty acids.
The values shown are estimated means. Significantly different entries for the GM cotton are marked with an asterisk.
(a): Cotton GHB119 treated with glufosinate ammonium-based herbicides.
(b): Cotton GHB119 treated only with conventional herbicides.

Based on the well-known biochemical role of the compounds in Tables 3 and 4 and on the reported ranges of natural variation described in the OECD consensus document (OECD, 2009) and published in the literature (e.g. Berberich et al., 1996), the GMO Panel identified no need for further assessment with regard to food and feed safety, except for crude protein and tocopherols.

Crude protein content in cotton GHB119 was reduced compared to its conventional counterpart in both field trial studies (Tables 3 and 4), and in the USA field trials it fell outside the range (21.8–34.2% DM) described in the literature (OECD, 2009). Crude protein content is considered for further nutritional assessment in Section 3.3.1.4.

Considering that cottonseed is a relevant source of vegetable oil due to its high tocopherol content, the reduced levels of tocopherols in cotton GHB119 compared to its conventional counterpart are considered for further nutritional assessment Section 3.3.1.4.

3.2.1.4. Effect of processing[31]

Processed products

Based on the outcome of the comparative assessment, processing of the cotton GHB119 into food and feed products is not expected to result in products being different from those of commercial non-GM cotton varieties.

Compositional data were obtained on processed products of ginned cottonseeds from an additional field trial carried out at one site in Argentina in 2008.[32] In this field trial, cottonseed samples of cotton GHB119 and Coker 315[33] treated with glufosinate ammonium-based herbicides were harvested and processed, at a pilot processing plant simulating industrial practice, into cotton products, including delinted seeds, hulls, meal (heated, flaked and extruded, solvent extraction), toasted meal, crude oil and refined bleached deodorised (RBD) oil.

Delinted seeds, meal and toasted meal were analysed for proximates, fibre, amino acids, minerals, α-tocopherol, free and total gossypol, cyclopropenoid fatty acids and phytic acid. Delinted seeds were

[31] Dossier: Part I – Section D7.6.
[32] Dossier: Part I – Section D7.6.
[33] Coker 315 has a very similar genetic background to Coker 312, hence it is considered an appropriate conventional counterpart for cotton GHB119; additional information: 4/7/2012.

also analysed for fatty acids. Hulls were analysed for proximates and fibre; crude and refined oils were analysed for fatty acids, tocopherols, total gossypol and cyclopropenoid fatty acids. All cottonseed fractions of cotton GHB119 showed lower protein content compared to its conventional counterpart.[34] The levels of cyclopropenoid fatty acids were lower in all feed fractions derived from cotton GHB119, including crude and RBD oil.

In the same processing study, the levels of the newly expressed proteins Cry2Ae and PAT were measured and detected in cotton GHB119-derived fractions but not in those from Coker 315. Cry2Ae and PAT were detectable in delinted cottonseeds, cottonseed meal (at very low levels in toasted meal) and hulls, but not detectable in crude and RBD oil from cotton GHB119.[35]

The values obtained for the composition of products derived from cotton GHB119 were similar to those of its conventional counterpart Coker 315 and within the values reported in literature (e.g. Forster and Calhoun, 1995; OECD, 2009).

Newly expressed proteins

Effect of temperature on newly expressed protein. The thermal stability of the bacterial Cry2Ae protein was evaluated after protein exposure at temperatures of 60, 75 and 90°C for periods of 10, 30 and 60 min, by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by Coomassie blue staining and western blot analyses. No significant changes in the Cry2Ae protein band intensity were observed after incubation at 60°C up to 60 min; a slight decrease in the Cry2Ae protein band intensity was observed after incubation at 75°C up to 60 min and at 90°C for 10 min; the Cry2Ae protein band intensity was markedly decreased after incubation at 90°C for 30 min, disappearing after 60 min.[36] The thermal stability of the Cry2Ae protein was also assessed in a bioassay conducted on a target insect species (*Heliothis zea*). After exposure of the Cry2Ae protein for 5, 10, 30, 60, 120, 240 min and overnight to temperatures of 45°C and 60°C, the protein was fed to *H. zea* to assess its activity. While the Cry2Ae protein remained stable at 45°C even during overnight incubation, the activity started decreasing after 60 min at 60°C, continuing to decrease with longer incubation times, up to sixfold after incubation over 240 min.[37]

3.2.2. Conclusion

The GMO Panel concluded that the differences identified in the agronomic and phenotypic characteristics between cottonseed GHB119 and its conventional counterpart did not require further assessment for food and feed safety. The differences are further assessed for their potential environmental impact in Section 3.4.1.1.

The differences identified between cotton GHB119 and its conventional counterpart in fuzzy cottonseed levels of crude protein and total tocopherol are further assessed in Section 3.3.1.4. The GMO Panel concluded that none of the other differences identified in the composition of fuzzy cottonseeds necessitated further assessment regarding food and feed safety.

The GMO Panel concluded that the compositional differences identified between products derived from cotton GHB119 and from its conventional counterpart did not require further assessment for food and feed safety.

3.3. Food/Feed safety assessment

3.3.1. Evaluation of relevant scientific data

3.3.1.1. Toxicology[38]

Cotton GHB119 expresses two new proteins, Cry2Ae and PAT (Section 3.1.1).

Proteins used for safety assessment

Given the technical restraints in producing large enough quantities for safety testing from plants, the Cry2Ae protein was recombinantly produced in *B. thuringiensis*. The purified protein from the

[34] Significantly different protein levels (in percentage dry matter (% DM), cotton GHB119 vs Coker 315) were as follows: 22.9 vs 25.8% DM in delinted cottonseed; 7.3 vs 8.1% DM in hulls; 33.2 vs 39.8% DM in cottonseed meal; 34.9 vs 38.7% DM in toasted cottonseed meal.
[35] Dossier: Part I – Section D7.7.
[36] Dossier: Part I – Section D7.8.1.
[37] Additional information: 2/3/2015.
[38] Dossier: Part I – Section D7.8.

bacterial source and from cotton GHB119 were characterised and compared in terms of their physicochemical, structural and functional properties.

a) Cry2Ae characterisation and equivalence. SDS-PAGE and western blot analysis showed that plant- and microbe-derived Cry2Ae proteins[39] had the expected molecular weight of ~ 71 kDa and were similarly immunoreactive to the Cry2Ae protein specific antibodies. Glycosylation detection analysis confirmed the absence of glycosylation for the Cry2Ae protein from cotton GHB119. Amino acid sequence analysis by liquid chromatography/mass spectrometry suggested that both plant- and microbe-derived proteins matched their expected sequence. At the N-terminus, the transit peptide of the plant form was cleaved at the expected residue. Functional equivalence was demonstrated by an insect bioassay and showed that both plant- and microbe-derived proteins had comparable biological activity. In addition, to justify the appropriateness of the recombinant protein batch used in the new 28-day oral toxicity study in mice[40] provided upon request of the GMO Panel, a comparison of this batch with the microbial Cry2Ae batch used in the original 28-day study[41] was provided; physical and chemical properties of the two batches were comparable.[42] SDS-PAGE and western blot analysis showed that microbe-derived Cry2Ae proteins from both batches had the expected molecular weight of ~ 71 kDa and were similarly immunoreactive to the Cry2Ae protein specific antibodies. The absence of glycosylation was confirmed. Amino acid sequence analysis by liquid chromatography/mass spectrometry and the N-terminal sequence obtained for both Cry2Ae protein batches were consistent with the theoretical amino acid sequence for the Cry2Ae protein.

Based on these data, the GMO Panel accepts the use of the Cry2Ae protein derived from *B. thuringiensis* in the safety studies.

b) PAT characterisation. SDS-PAGE and western blot analysis showed that the plant-derived PAT protein had the expected molecular weight of ~ 21 kDa and was immunoreactive to the PAT protein specific antibodies. Glycosylation detection analysis confirmed the absence of glycosylation. Sequence analysis by mass spectrometry and N-terminal sequencing showed that the protein matched its expected sequence. These data also showed that the first five amino acids from the N-terminus could not be determined. In contrast, the C-terminus fully matched the expected sequence. The enzymatic activity of the plant PAT protein was determined using a biochemical *in vitro* assay.

Toxicological assessment of newly expressed proteins

The PAT protein has been previously assessed by the GMO Panel (e.g. EFSA, 2006c; EFSA GMO Panel, 2013) and no safety concerns for humans and animals were identified. Updated bioinformatics analysis did not reveal similarities of the PAT protein to known toxins.[43] The GMO Panel is not aware of any new information that would change these conclusions. The GMO Panel concludes that the PAT protein does not raise safety concerns.

The Cry2Ae protein has never been assessed by the GMO Panel.

a) Bioinformatic studies[43]

Bioinformatic analyses of the amino acid sequences of the Cry2Ae protein expressed in cotton GHB119 revealed no relevant similarities to known proteins toxic to humans and animals (Section 3.1.1.2).

b) *In vitro* degradation studies[44]

The resistance of the Cry2Ae protein to degradation by pepsin was studied in solutions at pH 1.2. The integrity of the test protein in samples of the incubation mixture taken at various time points was analysed by SDS-PAGE gel electrophoresis followed by protein staining or by western blotting. No intact Cry2Ae protein was observed at the 5 min incubation time point. Low molecular weight fragments of 3–5 kDa in the protein-stained SDS-PAGE gel completely disappeared after 10 min of incubation. By western blot analysis, these fragments were not immunologically reactive to a polyclonal antibody against Cry2Ae.

The GMO Panel has previously evaluated the *in vitro* degradation study submitted to support this application (EFSA-GMO-NL-2011-96) and the safety of the PAT protein in the context of other applications (e.g. EFSA, 2006c; EFSA GMO Panel, 2013).

[39] Cry2Ae batches NB210806P25 and VMLV1041-1.
[40] Batch 1410_Cry2Ae.
[41] Batch VMLV1041-1.
[42] Additional information: 23/12/2015.
[43] Additional information: 16/6/2016.
[44] Dossier: Part I – Section 7.9.1; additional information: 2/3/2015.

c) Acute oral toxicity testing[45]

A bacterial-derived Cry2Ae protein was administrated by oral gavage at a dose of 2,000 mg/kg body weight (bw) to male and female OF1 mice. No effects related to the Cry2Ae protein were observed.

The GMO Panel is of the opinion that acute toxicity testing of the newly expressed proteins is of little additional value for the risk assessment of the repeated consumption of food and feed from GM plants by humans and animals.

d) 28-day repeated dose toxicity study

In the original application, the applicant provided a 28-day oral toxicity study in mice with the Cry2Ae protein.[45] The study was not accepted by the GMO Panel due to several limitations.[46] Upon request of the GMO Panel, the applicant provided a new 28-day oral toxicity study in mice with the recombinant Cry2Ae protein[37] described above. The study was conducted in alignment with OECD TG 407[47]; however, the GMO Panel noted that analysis of coagulation and bile acids was not performed. The study was in compliance with the principles of Good Laboratory Practice (GLP).

Groups of singly caged C57BL/6J mice (10/gender per group) were administered by gavage (20 mL/kg bw) the Cry2Ae protein at a targeted nominal dose of 1,000 mg/kg bw per day or the vehicle alone (control group). Both feed and water were provided *ad libitum*. During the study, all animals were checked at least daily for mortality or general clinical signs. Detailed physical examinations were performed at least weekly. Body weights and feed consumption were recorded at least weekly. Ophthalmoscopy was carried out before the start and at the end of the treatment. On study days 25 and 29, blood samples were taken for haematological and clinical chemistry analyses, respectively (animals were fasted overnight for the latter). At the end of the treatment period, all animals were sacrificed and underwent a detailed necropsy examination with selected organs weighed. Organs and tissues from all animals from the Cry2Ae-treated and control groups were subjected to a comprehensive histological examination. Descriptive statistics were calculated for each group and per time period for body weight change parameters, and for average food consumption per day parameters. All the endpoints were tested for homogeneity of variance with an *F*-test ($\alpha = 0.05$). The group means were then compared either with a *t*-test (if the *F*-test was not significant) or with a modified *t*-test (if the *F*-test was significant). For body weight and average food consumption per day parameters, in case the initial *F*-test was significant, the analysis was repeated on transformed data (log or square root transformation).

One female mouse given the Cry2Ae protein was found dead on day 26 of the study. This mouse showed at necropsy an abscess with skin ulceration in the ventral axillary area, microscopically associated with skin ulcerative and necrotising inflammation, and an enlarged spleen. These lesions were considered incidental in the study report. No clinically relevant findings or ophthalmic changes were observed. Statistically significantly lower body weight and cumulative body weight gain were seen on study day 22 in females given the Cry2Ae protein compared to the female controls; since this finding was transient and the final body weight did not differ, this was not considered toxicologically relevant. There were no statistically significant differences in haematology parameters in male animals. Females given the Cry2Ae protein, when compared to their controls, showed significantly lower red blood cell counts (10.08 ± 0.23 vs $10.33 \pm 0.24 \times 10^{12}$/L), haemoglobin concentration (15.40 ± 0.3 vs 15.92 ± 0.35 g/dL) and mean corpuscular haemoglobin concentration (29.32 ± 0.41 vs 29.75 ± 0.34 g/dL). The differences were minimal, not associated with changes in related parameters (e.g. reticulocyte count, spleen weight, gross pathology and histopathology, bone marrow histopathology) and were therefore considered not toxicologically relevant by the GMO Panel. Regarding clinical chemistry parameters, sodium levels were significantly lower in males given the Cry2Ae protein compared to their controls (158.3 ± 1.16 vs 160 ± 0.94 mmol/L); in females given the Cry2Ae protein, alkaline phosphatase activity (ALP) and urea concentrations were higher compared to the control (ALP: 164.8 ± 19.14 vs 148.4 ± 14.21 IU/L; urea: 15.36 ± 1.84 vs 12.77 ± 1.68 mmol/L). The differences were minimal, not associated with changes in related

[45] Dossier: Part I – Section 7.8.1.

[46] The WG was concerned about the single-dose group used, the low dose tested (75 mg/kg body weight (bw) per day) the absence of a vehicle control and the lack of a full set of pathology, including organ weights and histopathology.

[47] OECD (Organisation for Economic Co-operation and Development), Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents. OECD Guidelines for the Testing of Chemicals, Section 4.

parameters (e.g. liver and kidney weights, gross pathology and histopathology) and were therefore considered not toxicologically relevant by the GMO Panel. Significantly decreased thymus weight was found in males given the Cry2Ae protein compared to control males (0.0243 ± 0.004 g vs 0.0288 ± 0.003 g absolute weight; 0.1174 ± 0.017 vs 0.1374 ± 0.016% relative to body weight; 5.3735 ± 0.875 vs 6.2993 ± 0.703% relative to brain weight). The differences were minimal, not associated with changes in related parameters (e.g. lymphocytes count, thymus histopathology) and were therefore considered not toxicologically relevant by the GMO Panel. Macroscopic examinations at necropsy revealed no gross pathological findings related to the treatment with the Cry2Ae protein. Microscopic examinations of selected organs and tissues identified no treatment-related differences in the incidence and severity of histopathological findings between the groups.

Regarding the lack of some parameters requested by OECD TG407[47] (i.e. coagulation; bile acids), the GMO Panel considered that the integrated assessment of available related parameters (e.g. platelet count,[48] total bilirubin, alanine aminotransferase (ALT) and aspartate transaminase (AST) activity, liver weights, gross and histopathology) allowed to conclude in this study on coagulation and liver toxicological assessment.

The GMO Panel concludes that no adverse effects were observed after a 28-day administration of the Cry2Ae protein to mice at a dose of 1,000 mg/kg bw per day.

Toxicological assessment of new constituents other than proteins and/or changed levels of natural constituents

No new constituents other than the Cry2Ae and PAT proteins are expressed in cotton GHB119 and no relevant changes in the composition of the GM cotton were detected in the comparative compositional analysis (see Section 3.2.1.3).

3.3.1.2. Animal studies with the food/feed derived from GM plants

a) Rat subchronic study[49]

In a subchronic, 13-week feeding study, spontaneously submitted by the applicant, four groups of rats (strain Wistar Rj:WI (IOPS HAN), 10 male and 10 female animals per group) received diets containing toasted cottonseed meal: (i) 5% meal derived from cotton GHB119 (supplemented with 5% meal derived from the conventional counterpart, Coker 312), (ii) 10% meal from cotton GHB119, (iii) 10% meal from Coker 312 and (iv) 10% meal from commercial non-GM cotton (FM958), respectively.

Animals were housed in cages with five rats of the same gender per cage; however, in the statistical analysis, the individual animal was considered as the experimental unit, ignoring any possible bias due to cage interactions. Since the cage should be considered the experimental unit and due to the low number of experimental units per treatment (two per sex), the GMO Panel did not consider this study in its evaluation.

b) Chicken feeding study[49]

A total of 420 day-old Ross #708 broiler chickens of both genders was fed diets containing approximately 5% of toasted cottonseed meal from cotton GHB119 (test group, confirmed by PCR), the non-GM near-isogenic cottonseed meal (Coker 315[33], the conventional counterpart) and from a non-GM commercial cottonseed (FiberMax 958), respectively, for 42 days. The birds were randomly allocated by sex to pens (10 per cage), each treatment consisting of seven male pens and seven female pens.

Overall, mortality (including three culled birds) was high (about 9%) with no significant difference between the groups, and during the study, a total of 103 out of 420 birds exhibited clinical symptoms unrelated to treatment.[23] In addition, the GMO Panel observed that the diets used in the study were balanced with essential amino acids; e.g. the content of threonine and lysine in GM cottonseed diet was doubled in the grower diet as compared with the counterpart cottonseed grower. Because of these limitations, the GMO Panel could not use this study to conclude on the nutritional value of cottonseed meal from cotton GHB119.

[48] Tomlinson et al. (2013).
[49] Dossier: Part I – Section 7.8.4; additional information: 4/7/2012.

c) Northern bobwhite (*Colinus virginianus*) toxicity study[50]

The dietary toxicity study performed was based upon the US EPA OPPTS guideline 850.2200 (April 1996).[51] and the OECD guideline 205 (April 1984).[52] A total of 36 Northern bobwhite of indeterminate gender, 11 days old at experimental start, was fed diets containing approximately 10% toasted cottonseed meal from cotton GHB119 or 10% non-GM near-isogenic cottonseed meal from cotton Coker 312 or a non-GM commercial diet, respectively, for 5 days, followed by a 3-day post-exposure observation period where birds were fed the non-GM commercial diet only. This study was not designed for the risk assessment of feeds; therefore, the GMO Panel could not consider it for the nutritional evaluation or the assessment of potential unintended effects of cottonseed GHB119.

3.3.1.3. Allergenicity[53]

The strategies to assess the potential risk of allergenicity focus on the source of the recombinant protein, on the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons and on whether the transformation may have altered the allergenic properties of the modified plant.

a) Assessment of allergenicity of the newly expressed protein[54]

A weight-of-evidence approach was followed, taking into account all of the information obtained on the newly expressed protein, as no single piece of information or experimental method yield sufficient evidence to predict allergenicity (EFSA, 2006a; Codex Alimentarius, 2009; EFSA GMO Panel, 2011a).

The *cry2Ae* gene originates from *B. thuringiensis* subsp. *kurstaki*, a soil microorganism that is not considered to be an allergenic source. The source of the *bar* gene encoding for the PAT protein is *S. hygroscopicus*, which is also a soil microorganism and not considered to be an allergenic source.

Updated bioinformatic analyses[55] of the amino acid sequences of the Cry2Ae and PAT proteins, using the criterion of 35% identity in a sliding window of 80 amino acids, revealed no significant similarities to known allergens. In addition, the applicant performed analyses searching for matches of eight contiguous identical amino acid sequences between the Cry2Ae and PAT proteins and known allergens, which confirmed the outcome of the previous bioinformatic analysis.

The studies on resistance to degradation of the Cry2Ae and PAT proteins by pepsin have been described in Section 3.3.1.1.

The GMO Panel has previously evaluated the safety of the PAT protein in the context of several applications and no concerns on allergenicity were identified (e.g. EFSA, 2006c; EFSA GMO Panel, 2013).

There is no information available on the structure or function of the newly expressed Cry2Ae protein that would suggest an adjuvant effect due to its presence in cotton GHB119 resulting in or increasing an eventual immunoglobulin E (IgE) response to a bystander protein.

In the context of the present application, the GMO Panel considers that there are no indications that the newly expressed Cry2Ae and PAT proteins individually, or their simultaneous presence, in cotton GHB119 may be allergenic.

b) Assessment of allergenicity of GM plant products

The GMO Panel regularly reviews the available publications on food allergy to cottonseed (EFSA GMO Panel, 2016). However, to date, cotton has not been considered to be a common allergenic food[56] (OECD, 2009). Therefore, the GMO Panel did not request experimental data to analyse the allergen repertoire of GM cotton.

In the context of this application and considering the data from the molecular characterisation, the compositional analysis and the assessment of the newly expressed proteins (see Sections 3.1.1, 3.2.1

[50] Dossier: Part I – Section 7.8.4.
[51] United States Environmental Protection Agency, 1996 Office of Prevention, Pesticides and Toxic Substances. Ecological Effects Test Guideline, OPPTS 850.2200. Avian Dietary Toxicity Test. April 1996. US Environmental Protection Agency, Washington, DC.
[52] OECD (Organisation for Economic Co-operation and Development), 1984 OECD Guidelines for Testing of Chemicals, 205, Avian Dietary Toxicity Test. 10 pp.
[53] Dossier: Part I – Section D7.9.
[54] Dossier: Part I – Section D7.9.1; additional information: 11/11/2015, 2/3/2015 and 13/3/2013.
[55] Additional information: 16/6/2016.
[56] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

and 3.3.1.1), the GMO Panel identified no indications of a potentially increased allergenicity of food and feed derived from cotton GHB119 with respect to that derived from its conventional counterpart.

3.3.1.4. Nutritional assessment of GM food/feed[57,58]

The intended trait of cotton GHB119 is insect resistance and herbicide tolerance, with no intention to alter the nutritional parameters. The outcome of the compositional analysis (see Section 3.2.1.3) indicated that the introduction of food and feed products derived from cotton GHB119 into the food and feed supply is expected to have no adverse nutritional impact, as compared to its conventional counterpart.

The compositional data identified lower crude protein content in the GM cotton GHB119 compared to its conventional counterpart (Section 3.2.1.3, Tables 3 and 4). The main product for human consumption derived from cottonseed is the oil. Oil is not considered a source of protein for human nutrition, being almost devoid of protein contents. Products from cottonseed other than oil might be used for human consumption (e.g. processed linter pulp, fibre) generally in very small amount (OECD, 2009). The GMO Panel considers the lower crude protein content of the oil and of other products derived from cotton GHB119 to be of no concern as regards human nutrition. The GMO Panel considered the lower crude protein content in the GM cotton GHB119 of no safety concern, since a proper diet formulation can balance this endpoint.

The compositional data identified lower total tocopherol levels in seeds from cotton GHB119 compared to its conventional counterpart (101.7 and 107.0 mg/kg DM in cottonseeds from cotton GHB119, treated and not treated with the intended herbicides, respectively; 114.0 mg/kg DM in the conventional counterpart, Table 4). Detailed oil compositional analysis showed that the pattern of tocopherol fractions from cotton GHB119 oil is similar to that of the conventional counterpart, including α-tocopherol content.[59] Tocopherol content is a relevant quality parameter for vegetable oils including cottonseed oil. The GMO Panel considers that the changes in total tocopherol concentration in cotton GHB119 seeds are not of concern for human nutrition. The GMO Panel considered the lower total tocopherol levels in seeds from cotton GHB119 of no safety concern, since animal complete feeds are balanced with vitamins.

The GMO Panel concludes that cotton GHB119 is as nutritious as its conventional counterpart.

3.3.1.5. Post-market monitoring of GM food/feed

No biologically relevant compositional, agronomic and phenotypic changes were identified in cotton GHB119 when compared with its conventional counterpart. Furthermore, the overall intake or exposure is not expected to change because of the introduction of cotton GHB119 into the market. The GMO Panel therefore considers cotton GHB119 to be as safe as its conventional counterpart and that PMM (EFSA, 2006a; EFSA GMO Panel, 2011a) of the food/feed derived from cotton GHB119 is not necessary.

3.3.2. Conclusion

The safety assessment identified no concerns regarding the potential toxicity or allergenicity of the newly expressed Cry2Ae and PAT proteins in cotton GHB119, and found no indications of a potentially increased allergenicity of cotton GHB119 with respect to non-GM cotton. Based on the results of comparative analysis and on the nutritional assessment of crude protein and tocopherol content, the nutritional characteristics of food and feed derived from cotton GHB119 are not expected to differ from that of food and feed derived from non-GM cotton varieties, and therefore the GMO Panel concludes that cotton GHB119 is as safe and nutritious as its conventional counterpart.

3.4. Environmental risk assessment and monitoring plan

3.4.1. Evaluation of relevant scientific data

Considering the scope of application EFSA-GMO-NL-2011-96 (which excludes cultivation), the environmental risk assessment (ERA) of cotton GHB119 is mainly concerned with: (1) the exposure of bacteria to recombinant DNA in the gastrointestinal tract of animals fed GM material and bacteria present in the environments exposed to their faecal material (manure and faeces); and (2) the accidental release into the environment of viable cottonseeds GHB119 during transportation and processing (EFSA GMO Panel, 2010).

[57] Dossier: Part I – Section D7.2.5.
[58] Additional information: 3/2/2015.
[59] Dossier: Part I – Section D7.2.5; additional information: 28/6/2016 and 25/8/2016.

3.4.1.1. Environmental risk assessment

Persistence and invasiveness of the GM plant[18]

In southern Europe, *Gossypium herbaceum* and *G. hirsutum* have been grown since the 19th century and led to transient or locally naturalised cotton plants in the same area (Davis, 1967; Tutin et al., 1992; Sarno et al., 1993; Celesti-Grapow et al., 2010). However, survival of cottonseeds outside cultivation areas in Europe is limited due to the absence of a seed dormancy phase. Even if seeds from spillage germinate, the resulting cotton plants are unlikely to survive due to factors such as cold climatic conditions, the susceptibility to diseases and their low competitiveness (Eastick and Hearnden, 2006). For example, after the end of cotton cultivation in Italy in 1950s, no feral cotton was reported in southern Italy, except in some restricted areas (Sarno et al., 1993; Celesti-Grapow et al., 2010). Also in other cotton-growing regions, such as in Australia, surveys showed that feral GM cotton established infrequently along transportation routes and mostly as transient populations (Addison et al., 2007).

Cotton GHB119 has been developed for protection against certain lepidopteran pests, such as the CBW (*H. zea*), TBW (*H. virescens*) and FAW (*S. frugiperda*), and tolerance to glufosinate ammonium-based herbicides. The increased resistance against cotton insect pests due to the expression of the *cry2Ae* gene may provide a selective advantage in situations where plant survival is affected by pest pressure. Also, the *pat* gene coding for the herbicide tolerance trait can provide a potential agronomic and selective advantage for this GM cotton plant when glufosinate ammonium-based herbicides are applied. However, in case of accidental release into the environment of viable cottonseeds GHB119 during transportation and processing, establishment and survival of this GM cotton in the EU is limited by the biotic and abiotic factors described above.

As described in Section 3.2.1.2, GM cotton GHB119 treated with glufosinate ammonium-based herbicides and treated with conventional herbicides differed from its conventional counterpart in plant height, number of developed nodes, first bolls position, plant emergence, days until flowering, days until boll formation, seed number and weight and for seed and fibre yield. GM cotton GHB119 conventionally treated and treated with glufosinate ammonium-based herbicides differed from its conventional counterpart also for several fibre characteristics and, as expected, in biotic interactions, due to the expression of the toxic protein (Cry2Ae). Considering the biology of cotton, the differences observed in agronomic and phenotypic characteristics are unlikely to be biologically relevant in terms of increased fitness potential. In order to investigate additional characteristics of the GM cotton indicative of changes in survival, pollen morphology and germination were investigated, as well as the overwinter capacity of the cottonseeds GHB119. The conducted studies did not identify differences between the GM cotton and its conventional counterpart.

In the case of accidental release into the environment of cotton GHB119, there are no indications of an increased likelihood of establishment and spread of occasional feral cotton GHB119 plants. Should these plants be exposed to glufosinate ammonium-based herbicides, they are likely to exhibit a selective advantage that could increase their transient local occurrence. However, this will not result in different environmental impacts compared to conventional cotton.

In addition to the data presented by the applicant, the GMO Panel is not aware of any scientific report of increased spread and establishment of GM cotton GHB119 in regions where it is cultivated, and of any change in survival capacity, including overwintering.

The GMO Panel concludes that it is very unlikely that cotton GHB119 will differ from conventional cotton varieties in its ability to survive or establish feral populations under European environmental conditions.

Effects of gene transfer[60]

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer of DNA, or through vertical gene flow via seed dispersal and cross-pollination from feral plants originating from spilled seeds.

a) Plant-to-bacteria microorganism gene transfer

Genomic DNA is a component of many food and feed products derived from cotton. It is well documented that DNA present in food and feed becomes substantially degraded during processing and digestion in the human or animal gastrointestinal tract. However, a low level of exposure of fragments

[60] Dossier: Part I – Section D6.

of ingested DNA, including the recombinant fraction of such DNA, to bacteria in the digestive tract of humans, domesticated animals and other environments exposed to the GM plant or plant material is expected. Current scientific knowledge of recombination processes in bacteria suggests that horizontal transfer of non-mobile, chromosomally located DNA fragments between unrelated organisms (such as from plants to bacteria) is not likely to occur at detectable frequencies under natural conditions (for further details, see EFSA, 2009).

A successful horizontal gene transfer would require stable insertion of the recombinant DNA sequences into a bacterial genome and a selective advantage to be conferred to the transformed host. The only mechanism known to facilitate horizontal transfer of non-mobile, chromosomal DNA fragments to bacterial genomes is homologous recombination. This requires the presence of stretches of DNA sequences that are similar in the recombining DNA molecules. The similarity between the plant and bacterial sequences can be situated in the coding region of a recombinant protein (transgene) or in the border regions of the recombinant gene cassettes inserted into the plant genome. In the case of sequence identity with the transgene itself, recombination would result in gene replacement. In the case of identity with border regions, recombination could result in the insertion of additional DNA sequences in bacteria and thus confer the potential for new properties.

Cotton GHB119 contains genetic elements with identity or high similarity to those of bacteria. These are the coding sequence of *cry2Ae*, a codon-optimised sequence of *cry2Ae* from *B. thuringiensis*, and the coding sequence of the phosphinothricin acetyltransferase (*bar*) gene of *S. hygroscopicus* with a minor modification. The flanking regions of the recombinant gene insert contain approximately 20 bp long sequences of the truncated right and left border of the Ti-plasmid of *A. tumefaciens*. Both species, *B. thuringiensis* and *S. hygroscopicus*, are not considered to be prevalent in the main receiving environment, i.e. the gastrointestinal tract of humans or animals. Both occur in soil and, in addition, *B. thuringiensis* has been frequently isolated from the guts of insects (Jensen et al., 2003). However, occurrence of the recombinant genes outside their immediate receiving environment in the habitats of both bacterial species cannot be ruled out (Hart et al., 2009) and is therefore also considered here.

On a theoretical basis (i.e. without any study providing experimental evidence for horizontal gene transfer (HGT) in the case of GM food and feed derived from cotton GHB119 or any other GM plant), it can be assumed that, as an extremely rare event, homologous recombination can occur between the recombinant *bar* gene and its natural variant in *S. hygroscopicus* or other Actinobacteria. Such recombination events would only replace natural variants (substitutive recombination) and are therefore are unlikely to provide any new property connected to a selective advantage for the recipient organisms (EFSA, 2009). The *cry2Ae* gene, being codon optimised, has insufficient similarity to known corresponding bacterial genes to facilitate homologous recombination between GM cotton and environmental bacteria.

Double homologous recombination of the flanking regions with those on Ti-plasmids of *A. tumefaciens* were not considered because the 20 bp flanking regions for the Ti-plasmid would not be of sufficient lengths to facilitate double homologous recombination.

In addition to homology-based recombination processes, illegitimate recombination that does not require DNA similarity between the recombining DNA molecules is theoretically possible. However, the transformation rates for illegitimate recombination are considered to be 10^{10}-fold lower than for homologous recombination (Hülter and Wackernagel, 2008; EFSA, 2009). Illegitimate recombination events have not been detected in studies that have exposed bacteria to high concentrations of GM plant DNA (EFSA, 2009). Thus, this process, in comparison with homologous recombination, is not considered to be a minimal contributor to HGT events. In comparison with the above-described homology-facilitated recombination processes, the contribution of illegitimate recombination is extremely low.

The GMO Panel concludes that the *bar* gene from cotton GHB119 may, on a theoretical basis, replace similar genes by homologous recombination with environmentally present *S. hygroscopicus* or other Actinobacteria with corresponding genes. Such a transfer would not provide a new selective advantage to the recipients. Codon optimisation altered the *cry2Ae* gene so that homologous recombination with corresponding bacterial genes would not be facilitated. Thus, considering the scope of this application, the GMO Panel has not identified any concern associated with HGT from cotton GHB119 to bacteria.

b) Plant-to-plant gene transfer

Considering the scope of application EFSA-GMO-NL-2011-96 and the biology of cotton, a pathway to possible harm pertains to the potential of occasional feral GM cotton plants originating from accidental spillage of imported cottonseed GHB119 to transfer recombinant DNA to sexually cross-compatible plants.

Cotton is predominantly an annual self-pollinating crop, although cross-pollination can occur at low frequencies in the presence of insect pollinators (such as wild bees, honeybees, bumblebees) (OECD, 2008).

The extent of cross-pollination will mainly depend on the scale of accidental release during transportation and processing, and the successful establishment and subsequent flowering of GM cotton plants. For cotton, no wild relatives have been reported in Europe, therefore any vertical gene transfer is limited to cultivated (*G. hirsutum* and *G. herbaceum*) and feral cotton plants. However, gene transfer to *G. herbaceum* is considered unlikely due to the difference in ploidy level.

The occurrence of feral GM cotton is expected to be limited. For plant-to-plant gene transfer to occur, imported cottonseeds need to be processed outside the importing ports, transported into regions of cotton production in Europe, spilled during transportation, germinate and develop into plants in the very close vicinity of cotton fields, and there needs to be an overlap of flowering periods and environmental conditions favouring cross-pollination. It must be noted that most cottonseeds are processed in the countries of production or in ports of importation.

In conclusion, the GMO Panel considers that the likelihood of environmental effects as a consequence of the spread of genes from cotton GHB119 in Europe will not differ from that of conventional cotton, even after exposure to glufosinate ammonium-based herbicides.

Interactions of the GM plant with target organisms[61]

Considering the scope of application EFSA-GMO-NL-2011-96, potential interactions of occasional feral cotton GHB119 plants arising from seed import spills with target organisms are not considered a relevant issue by the GMO Panel.

Interactions of the GM plant with non-target organisms[62]

Considering the scope of application EFSA-GMO-NL-2011-96 and the low level of exposure to the environment, potential interactions of the GM cottonseeds or plants arising from spillage of imported seeds with non-target organisms were not considered a relevant issue by the GMO Panel.

The GMO Panel evaluated whether the expressed Cry2Ae protein might potentially affect non-target organisms by entering the environment through faecal material of animals fed GM cottonseed products. Cry proteins are degraded by enzymatic activity in the gastrointestinal tract, meaning that only low amounts of Cry proteins would remain intact to pass out in faeces to the soil. This was demonstrated for Cry1Ab (Einspanier et al., 2004; Lutz et al., 2005, 2006; Wiedemann et al., 2006; Guertler et al., 2008; Paul et al., 2010). Further degradation of the proteins in the manure and faeces would take place because of microbiological proteolytic activity. In addition, there would be further degradation of Cry proteins in soil, reducing the possibility for exposure of potentially sensitive non-target organisms. Although Cry proteins may bind to clay minerals and organic substances in soil, thereby reducing their availability to microorganisms for degradation, there are no indications of persistence and accumulation of Cry proteins from GM crops in soil (Gruber et al., 2011; Valldor et al., 2015). The GMO Panel is not aware of evidence of released Cry proteins from GM plants causing significant negative effects on soil microorganisms.

Considering the scope of the application, it can be concluded that the exposure of potentially sensitive non-target organisms to the Cry2Ae protein is likely to be very low and of no biological relevance.

Interactions with the abiotic environment and biochemical cycles[63]

Considering the scope of application EFSA-GMO-NL-2011-96, and the low level of exposure to the environment, potential interactions with the abiotic environment and biogeochemical cycles were not considered a relevant issue by the GMO Panel.

3.4.2. Post-market environmental monitoring[64]

The objectives of a post-market environmental monitoring (PMEM) plan, according to Annex VII of Directive 2001/18/EC, are (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the ERA are correct and (2) to identify the

[61] Dossier: Part I – Section D8 and D9.4.
[62] Dossier: Part I – Section D9.5.
[63] Dossier: Part I – Section D9.8 and D10.
[64] Dossier: Part I – Section D11.

occurrence of adverse effects of the GMO, or its use, on human health or the environment that were not anticipated in the ERA.

Monitoring is related to risk management, and thus, a final adoption of the PMEM plan falls outside the mandate of EFSA. However, the GMO Panel gives its opinion on the scientific content of the PMEM plan provided by the applicant (EFSA, 2006b; EFSA GMO Panel, 2011b). The potential exposure to the environment of cotton GHB119 would be through faecal material from animals fed the GM cotton or through accidental release into the environment of viable GM cotton seeds during transportation and processing.

The PMEM plan proposed by the applicant includes (1) the description of an approach involving operators (federations involved in cotton import and processing) reporting any observed adverse effect (s) of the GMO on human health and the environment to the applicants via a centralised system; (2) a coordinating system established by EuropaBio for the collection of the information recorded by the various operators; and (3) the use of networks of existing surveillance systems (Lecoq et al., 2007; Windels et al., 2008). The applicant proposes to submit a PMEM report on an annual basis and a final report at the end of the consent period.

The GMO Panel is of the opinion that the PMEM plan proposed by the applicant is in line with the scope of application EFSA-GMO-NL-2011-96. As no potential adverse environmental effects were identified, case-specific monitoring was not considered necessary. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

3.4.3. Conclusion

No safety concerns with regard to the environment from the import and processing of cotton GHB119 were identified. There are no indications of an increased likelihood of the establishment and spread of occasional feral cotton GHB119 plants in the case of accidental release into the environment of viable GM cottonseeds even when exposed to glufosinate ammonium-based herbicides. The unlikely, but theoretically possible, horizontal transfer of recombinant genes from cotton GHB119 to bacteria does not raise any environmental safety concern. Considering the scope of the application, potential interactions of cotton GHB119 with the biotic and abiotic environment were not considered a relevant issue by the GMO Panel. The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of cotton GHB119 and the GMO Panel guidelines on the post-market environmental monitoring of GM plants (EFSA, 2006b, EFSA GMO Panel, 2011b).

4. Overall conclusions and recommendations

The GMO Panel was asked to carry out a scientific assessment of cotton GHB119 for import, processing, and food and feed uses in accordance with Regulation (EC) No 1829/2003.

The molecular characterisation data establish that cotton GHB119 contains a single insert consisting of a single copy of the T-DNA containing the *cry2Ae* and the *bar* expression cassettes. No other parts of the plasmid used for transformation are present in cotton GHB119. Bioinformatic analyses and genetic stability studies did not raise safety issues. The levels of the Cry2Ae and PAT proteins in cotton GHB119 have been sufficiently analysed.

The safety assessment identified no concerns regarding the potential toxicity or allergenicity of the newly expressed Cry2Ae and PAT proteins in cotton GHB119, and found no indications of a potentially increased allergenicity of cotton GHB119 with respect to non-GM cotton. Based on the results of comparative analysis and on the nutritional assessment of crude protein and tocopherol content, the nutritional characteristics of food and feed derived from cotton GHB119 are not expected to differ from that of food and feed derived from non-GM cotton varieties. The GMO Panel concludes that cotton GHB119 is as safe and nutritious as its conventional counterpart. The GMO Panel considers that PMM of food/feed derived from cotton GHB119 is not necessary, given the absence of safety concerns identified.

Considering the scope of cotton GHB119, which excludes cultivation, there is no requirement for a scientific assessment of possible environmental effects associated with the cultivation of this GM cotton. In the case of accidental release into the environment of viable seeds of cotton GHB119 (e.g. during transport and processing), there are no indications of an increased likelihood of establishment and spread of feral cotton plants. The low level of environmental exposure of these GM cotton plants indicates that the risk to non-target organisms is extremely low. In the context of its intended uses, the unlikely but theoretically possible transfer of the recombinant gene from cotton GHB119 to environmental bacteria does not raise safety concern owing to the lack of a selective advantage which

would be conferred. The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of cotton GHB119 and the guidance document of the GMO Panel on post-market environmental monitoring of GM plants (EFSA GMO Panel, 2011b).

In conclusion, the GMO Panel considers that the information available for cotton GHB119 addresses the scientific comments raised by the Member States and that cotton GHB119, as described in this application, is as safe as its conventional counterpart with respect to potential effects on human and animal health and the environment in the context of the scope of this application.

Documentation provided to EFSA

1) Letter from the Competent Authority of The Netherlands received on 7 April 2011 concerning a request for the placing on the market of genetically modified cotton GHB119 submitted under Regulation (EC) No 1829/2003 by Bayer CropScience AG (EFSA reference EFSA-GMO-NL-2011-96).
2) Acknowledgement letter dated 27 April 2011 from EFSA to the Competent Authority of the Netherlands.
3) Letter from EFSA to the applicant dated 26 May 2011 requesting additional information under completeness check.
4) Letter from the applicant received on 30 August 2011 providing additional information under completeness check.
5) Letter from EFSA to the applicant dated 19 September 2011 requesting additional information under completeness check.
6) Letter from the applicant received on 31 October 2011 providing additional information under completeness check.
7) Letter from EFSA to the applicant dated 21 November 2011 delivering the 'Statement of Validity' of application EFSA-GMO-NL-2011-96 for the placing on the market of genetically modified cotton GHB119 submitted under Regulation (EC) No 1829/2003 by Bayer CropScience AG.
8) Letter from the applicant received on 25 November 2011 providing EFSA with an updated version of the application EFSA-GMO-NL-2011-96 submitted under Regulation (EC) No 1829/2003 by Bayer CropScience AG.
9) Letter from EFSA to the applicant dated 14 February 2012 requesting additional information and stopping the clock.
10) Letter from EFSA to applicant dated 17 April 2012 requesting additional information and maintaining the clock stopped.
11) Letter from applicant to EFSA received on 21 May 2012 providing additional information.
12) Letter from the applicant to EFSA received on 4 July 2012 providing additional information.
13) Letter from EFSA to the applicant dated 9 January 2013 re-starting the clock.
14) Letter from EFSA to the applicant dated 11 February 2013 requesting additional information and stopping the clock.
15) Letter from EFSA to the applicant dated 22 February 2013 requesting additional information and maintaining the clock stopped.
16) Corrigendum letter from EFSA to the applicant dated 28 February 2013 requesting complementary information and maintaining the clock stopped.
17) Letter from applicant to EFSA received on 13 March 2013 providing additional information.
18) Letter from applicant to EFSA received on 5 April 2013 providing additional information.
19) Letter from EFSA to the applicant dated 24 July 2013 requesting additional information and maintaining the clock stopped.
20) Letter from applicant to EFSA received on 2 March 2015 providing additional information.
21) Letter from EFSA to the applicant dated 24 June 2015 requesting additional information and maintaining the clock stopped.
22) Letter from applicant to EFSA received on 9 July 2015 providing additional information.
23) Letter from EFSA to the applicant dated 5 October 2015 requesting additional information and maintaining the clock stopped.
24) Letter from EFSA to the applicant dated 12 October 2015 requesting additional information and maintaining the clock stopped.
25) Letter from applicant to EFSA received on 11 November 2015 providing additional information.

26) Letter from EFSA to the applicant dated 26 November 2015 requesting additional information and maintaining the clock stopped.
27) Letter from applicant to EFSA received on 30 November 2015 providing additional information.
28) Letter from applicant to EFSA received on 23 December 2015 providing additional information.
29) Letter from EFSA to the applicant dated 16 February 2016 requesting additional information and maintaining the clock stopped.
30) Letter from applicant to EFSA received on 4 April 2016 providing additional information.
31) Email from EFSA to applicant dated 5 April 2016 re-starting the clock from 4 April 2016.
32) Letter from EFSA to the applicant dated 7 April 2016 requesting additional information and stopping the clock.
33) Letter from EFSA to the applicant dated 13 June 2016 requesting additional information and maintaining the clock stopped.
34) Letter from applicant to EFSA received on 16 June 2016 providing additional information.
35) Letter from applicant to EFSA received on 29 June 2016 providing additional information.
36) Email from EFSA to applicant dated 30 June 2016 re-starting the clock from 29 June 2016.
37) Letter from EFSA to the applicant dated 3 August 2016 requesting additional information and stopping the clock.
38) Letter from applicant to EFSA received on 25 August 2016 providing additional information.
39) Email from EFSA to applicant dated 26 August 2016 re-starting the clock from 25 August 2016.

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Abbreviations

AA	amino acid
aadA	aminoglycoside adenyltransferase
ADF	acid detergent fibre

ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANOVA	analysis of variance
AST	aspartate transaminase
ATCC	American Type Culture Collection
CBW	cotton bollworm
DM	dry matter
ELISA	enzyme-linked immunosorbent assay
ERA	environmental risk assessment
FA	fatty acid
FAW	fall armyworm larvae
FW	fresh weight
GLP	Good Laboratory Practice
GM	genetically modified
GMO	Genetically Modified Organisms
GMO Panel	EFSA Panel on Genetically Modified Organisms
HGT	horizontal gene transfer
IgE	immunoglobulin E
NDF	neutral detergent fibre
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PAT	phosphinothricin acetyltransferase
PCR	polymerase chain reaction
PMEM	post-market environmental monitoring
PMM	post-market monitoring
RBD	refined bleached deodorised
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TBW	tobacco budworm