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SCIENTIFIC OPINION

Scientific Opinion on application (EFSA-GMO-NL-2010-77) for the placing on the market of herbicide-tolerant genetically modified cotton GHB614 × LLCotton25 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience[1]

EFSA Panel on Genetically Modified Organisms (GMO)[2,3]

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ABSTRACT

Cotton GHB614 × LLCotton25 was produced by conventional crossing. The EFSA GMO Panel previously assessed the two single cotton events GHB614 and LLCotton25 and did not identify safety concerns. Integrity of the inserts was retained in the two-event stack cotton. No differences requiring further food and feed safety assessment were identified in the compositional analysis of cotton GHB614 × LLCotton25 except for a higher level of gossypol. The EFSA GMO Panel further assessed this difference and considers that it is of no safety relevance for animals and humans. Expression analysis and safety assessment of the newly expressed proteins identified no concerns regarding their potential toxicity and allergenicity. No indications of safety issues regarding the overall allergenicity of cotton GHB614 × LLCotton25 were identified. There are no indications of an increased likelihood of establishment and spread of feral cotton plants. Considering the scope of the application, potential interactions of cotton GHB614 × LLCotton25 with the biotic and abiotic environment were not considered a relevant issue. Environmental risks associated with an unlikely but theoretically possible horizontal gene transfer from cotton GHB614 × LLCotton25 to bacteria have not been identified. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of cotton GHB614 × LLCotton25.

KEY WORDS

GMO, cotton (*Gossypium hirsutum* L. and *Gossypium barbadense* L.), GHB614 × LLCotton25, herbicide tolerant, stack, Regulation (EC) No 1829/2003

[1] On request from the Competent Authority of the Netherlands for an application (EFSA-GMO-NL-2010-77) submitted by Bayer CropScience, Question No EFSA-Q-2010-00106, adopted on 11 April 2014.

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[3] Acknowledgement: The Panel wishes to thank the members of the Working Groups on Molecular Characterisation, Food and Feed Risk Assessment and Environmental Risk Assessment for the preparatory work on this scientific opinion; and EFSA staff: Hermann Broll, Antonio Fernandez Dumont, Ana Gomes and Sylvie Mestdagh for the support provided to this scientific opinion.

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SUMMARY

Following the submission of an application (EFSA-GMO-NL-2010-77) under Regulation (EC) No 1829/2003 from Bayer CropScience, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) was asked to deliver a scientific opinion on the safety of herbicide-tolerant genetically modified (GM) cotton GHB614 × LLCotton25.

The scope of application EFSA-GMO-NL-2010-77 is for food and feed uses, import and processing, but excludes cultivation within the European Union (EU).

In delivering its scientific opinion, the EFSA GMO Panel considered the application EFSA-GMO-NL-2010-77, the additional information provided by the applicant, the scientific comments submitted by the Member States and the relevant scientific publications. Application EFSA-GMO-NL-2010-77 was evaluated in accordance with the applicable guidance document at the time of its submission (EFSA, 2007). Wherever feasible, the principles described in currently used guidance documents (EFSA, 2010, 2011a) were taken into account. Previous assessment of the two single cotton events (GHB614 and LLCotton25) formed the basis for the evaluation of cotton GHB614 × LLCotton25. The risk assessment of this two-event stack cotton focused mainly on issues related to (a) stability, (b) expression of the events and (c) potential interactions between the events (EFSA, 2007). The scientific evaluation of the risk assessment included the molecular characterisation of the inserted DNA and the analysis of the expression of the new proteins. An evaluation of the comparative analyses of compositional, agronomic and phenotypic characteristics was undertaken, and the safety of the newly expressed proteins and the whole food/feed was evaluated with respect to potential toxicity, allergenicity and nutritional wholesomeness. Evaluation of environmental impacts and of the post-market environmental monitoring plan was also undertaken.

Cotton GHB614 × LLCotton25 was produced by conventional crossing of inbred lines of cotton GHB614 and cotton LLCotton25, combining tolerances to glyphosate and glufosinate-ammonium herbicides. The newly expressed proteins 2mEPSPS (expressed in cotton GHB614) and phosphinothricin acetyltransferase (PAT) (expressed in cotton LLCotton25) were assessed previously and no concerns were identified.

Molecular analysis confirmed that the integrity of cotton GHB614 and cotton LLCotton25 inserts was retained in the two-event stack cotton. Results of the updated bioinformatics analyses of the flanking sequences and the open reading frames spanning the insert–genomic DNA junctions did not indicate safety issues. The molecular characterisation of the single cotton events GHB614 and LLCotton25 did not indicate safety issues related to the possible interaction of these events within the two-event stack cotton. Considering the scope of the application, levels of the newly expressed proteins 2mEPSPS and PAT in seed produced from the two-event stack cotton were evaluated and found to be comparable to those from the single cotton events.

Based on the results of the agronomic, phenotypic and compositional analysis of samples from a representative range of environments, the EFSA GMO Panel concludes that no differences requiring further assessment with regard to safety were identified in the composition of cotton GHB614 × LLCotton25 except for the elevated level of gossypol in comparison with its conventional counterpart. The observed gossypol levels in cotton GHB614 × LLCotton25 were at the upper limit of ranges reported in literature. The elevated levels of gossypol were considered not to pose concerns for human or animal safety. The EFSA GMO Panel, after considering all the data for cotton GHB614 × LLCotton25 and for the newly expressed proteins 2mEPSPS and PAT, is of the opinion that interactions impacting the food and feed safety of cotton GHB614 × LLCotton25 are unlikely. The EFSA GMO Panel also concludes that cottonseed of GHB614 × LLCotton25 is as nutritious as its conventional counterpart.

The scope of application EFSA-GMO-NL-2010-77 is for food and feed uses, import and processing and does not include cultivation. Therefore, there is no requirement for scientific information on

possible environmental effects associated with the cultivation of cotton GHB614 × LLCotton25 in Europe. There are no indications of an increased likelihood of establishment and spread of feral cotton plants in case of accidental release into the environment of viable cotton GHB614 × LLCotton25 grains during transportation and processing for food and feed uses. Taking into account the scope of the application, both the rare occurrence of feral cotton plants and the low levels of exposure through other routes indicate that the risk to non-target organisms is extremely low. The unlikely but theoretically possible transfer of the recombinant gene from cotton GHB614 × LLCotton25 to environmental bacteria does not raise safety concerns owing to the lack of a selective advantage, which would be conferred. The scope of the post-market environmental monitoring plan and the reporting intervals proposed by the applicant are in line with the intended uses of cotton GHB614 × LLCotton25.

In conclusion, the EFSA GMO Panel is of the opinion that cotton GHB614 × LLCotton25 is as safe and as nutritious as its conventional counterpart in the context of its intended uses. The EFSA GMO Panel does not recommend a post-market monitoring for GM food and feeds, and the post-market environmental monitoring plan and reporting intervals are in line with the intended uses of the two-event stack cotton.

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BACKGROUND

On 4 February 2010, the European Food Safety Authority (EFSA) received from the Competent Authority of The Netherlands an application (Reference EFSA-GMO-NL-2010-77), for authorisation of genetically modified (GM) cotton GHB614 × LLCotton25 submitted by Bayer CropScience within the framework of Regulation (EC) No 1829/2003[4] on genetically modified food and feed for food and feed uses, import and processing.

After receiving the application EFSA-GMO-NL-2010-77 and in accordance with Articles 5(2)(b) and 17(2)(b) of Regulation (EC) No 1829/2003, EFSA informed Member States and the European Commission, and made the summary of the application available to the public on the EFSA website.[5] EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of Regulation (EC) No 1829/2003. On 3 August 2010, 11 November 2010 and 3 January 2011, EFSA received additional information (requested on 17 March 2010, 23 August 2010 and 6 December 2010, respectively). On 26 January 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 Member States and the European Commission, and consulted nominated risk assessment bodies of Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC[6] following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Member States had three months after the date of receipt of the valid application (until 26 April 2011) to make their opinion known.

The scope defined by the applicant at the time of submission was "*includes all food and feed products containing, consisting or produced from cotton GHB614 × LLCotton25 including products from inbreds and hybrids obtained by conventional breeding of this stacked cotton product. The application also covers the import and industrial processing of cotton GHB614 × LLCotton25 for all potential uses as any other cotton.*"

The EFSA GMO Panel carried out an evaluation of the scientific risk assessment of the GM cotton GHB614 × LLCotton25. On 15 April 2011, 16 September 2011 and 25 November 2013, the EFSA GMO Panel requested additional information. The applicant provided the requested information on 12 May 2011, 15 March 2013 and 20 December 2013, respectively.

In giving its scientific opinion 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 six months from the acknowledgement of the valid application. As additional information was requested by the EFSA GMO Panel, the time limit of six 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, the EFSA opinion shall include a report describing the assessment of the food and feed and stating the reasons for its opinion and the information on which its opinion is based. This document 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).

[4] 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.

[5] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2009-00444

[6] 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, 12.03.2001, p. 1-38.

TERMS OF REFERENCE

The EFSA GMO Panel was requested to carry out a scientific risk assessment of cotton GHB614 × LLCotton25 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 requirements based on the outcome of the risk assessment and, in the case of GMOs or food/feed containing or consisting of GMOs, conditions for the protection of particular ecosystems/environment 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 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.

ASSESSMENT

1. INTRODUCTION

The scope of application EFSA-GMO-NL-2010-77 is for food and feed uses, import and processing of all derived products of cotton GHB614 × LLCotton25 such as the production of refined oil from seeds, production of cellulose from linters as food or food ingredients and use of cotton seed meal and hulls in animal feed.

Cotton GHB614 × LLCotton25 was obtained by conventional crossing of two transgenic lines containing the single events (cotton transformation event GHB614, OECD unique identifier code BCS-GHØØ2-5 and transformation event LLCotton25, OECD unique identifier code ACS-GHØØ1-3).

It was developed to confer tolerance to glyphosate herbicides—inherited from its parental line GHB614 and achieved by expressing the modified enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (2mEPSPS)—and tolerance to glufosinate ammonium herbicides, inherited from its parental line LLCotton25 and achieved by expressing the enzyme phosphinothricin acetyl-transferase (PAT).

The genus *Gossypium* consists of more than 50 species, two of which are the most commonly cultivated species (*Gossypium hirsutum* and *G. barbadense*). The composition of cottonseed from *G. barbadense* does not differ from that of seed from *G. hirsutum* to an extent that a food and feed risk assessment of one species would not be applicable also to the other. Therefore, the risk assessment considered in this opinion is applicable to both *G. hirsutum* and *G. barbadense*.

Both single cotton events GHB614 and LLCotton25 have been assessed previously (EFSA, 2006c, 2009a) on the basis of experimental data (Table 1). No concerns were identified for human and animal health and the environment.

Table 1: Single cotton events already assessed by the EFSA GMO Panel

Event	Application	EFSA Scientific Opinion
GHB614	EFSA-GMO-NL-2008-51	2009
LLCotton25	EFSA-GMO-NL-2005-13	2006

2. ISSUES RAISED BY MEMBER STATES

Issues raised by Member States have been considered in this EFSA GMO Panel scientific opinion and are addressed in detail in Annex G of the EFSA overall opinion.[7]

3. UPDATED INFORMATION ON SINGLE EVENTS

No new safety-relevant scientific information regarding the two single cotton events was identified in a literature review[8] covering the period since the publication of the scientific opinions.

Updated bioinformatics-supported comparison of the amino acid sequence of the newly expressed proteins revealed no significant similarities to known toxins or allergens. The similarity search for allergens used the criterion of 35 % identity in a window of 80 amino acids. A search for matches of eight contiguous identical amino acid sequences between these proteins and known allergens was also made. The bioinformatics analyses on the junction regions for events GHB614 and LLCotton25 confirmed that no known endogenous genes were disrupted by any inserts. No biologically relevant

[7] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question= EFSA-Q-2010-00106
[8] Additional information, March 2013.

similarities to allergens or toxins for any of the putative peptides that might be produced from open reading frames (ORFs) within the inserts or spanning the junction regions were identified.[9]

Consequently, the EFSA GMO Panel considers that its previous conclusions on the safety of the single cotton events remain valid.

4. RISK ASSESSMENT OF THE TWO-EVENT STACK COTTON GHB614 × LLCOTTON25

4.1. Molecular characterisation

The characterisation of stacked events focuses on stability, expression and the potential for interactions between the events included in the stack. Such interactions may occur at different levels—DNA, RNA, protein and associated functions—and involve both the DNA inserts and the genomic DNA flanking the inserts. For the purpose of risk assessment, within the molecular characterisation, the consequences of such interactions are addressed by analysing the integrity of the events, i.e. the structure of the inserts and flanking DNA (Section 4.1.2) and the levels of the newly expressed proteins (Section 4.1.3). Considerations are also given to the possible interactions between the known biological functions conferred by the individual inserts.

4.1.1. Genetic elements and biological functions of the inserts[10]

Cotton GHB614 and LLCotton25 are combined by conventional crossing to produce the two-event stack cotton GHB614 × LLCotton25. The structures of the inserts introduced into the two-event stack cotton are described in detail in the relevant EFSA scientific opinions listed in Table 1. No new genetic modifications were involved. Genetic elements in the expression cassettes of the single events are summarised in Table 2.

Table 2: Genetic elements in the expression cassettes of the events stacked in cotton GHB614 × LLCotton25

Event	Promoter	5′ Leader	Transit peptide	Coding region	Terminator
GHB614	*Ph4a748At* (*Arabidopsis thaliana*)	intron1 h3At (*A. thaliana*)	TPotpC (*Zea mays*, *elianthus annuus*)	*2mepsps* (*Z. mays*)	3′ histone H4 At (*A. thaliana*)
LLCotton25	35S (CaMV)	–	–	*bar* (*Streptomyces hygroscopicus*)	*nos* (*Agrobacterium tumefaciens*)

Biological functions conferred by the inserts in cotton GHB614 × LLCotton25 are summarised in Table 3.

[9] Additional information, December 2013.
[10] Dossier: Part I—Section C1.

Table 3: Biological functions of the events stacked in cotton GHB614 × LLCotton25

Event	Protein	Function in donor organism	Function in GM plant
GHB614	2mEPSPS	Donor organism: *Zea mays* 5-Enolpyruvyl-shikimate-3-phosphate (EPSPS) synthase is an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Herrmann, 1995)	Two amino acid substitutions (2mEPSPS) result in lower affinity of the enzyme towards the herbicide glyphosate, conferring tolerance to glyphosate-based herbicides on the GM plant (Herouet-Guicheney et al., 2009; Garg et al., 2014)
LLCotton25	PAT	Donor organism: *Streptomyces hygroscopicus* ATCC21705 Phosphinothricin-acetyl-transferase (PAT) confers resistance to the antibiotic bialaphos (Thompson et al., 1987)	PAT acetylates L-glufosinate-ammonium and thereby confers tolerance to glufosinate ammonium-based herbicides (Droge-Laser et al., 1994)

The newly expressed enzymes participate in different metabolic pathways and no functional interactions are expected.

4.1.2. Integrity of the events in the two-event stack

The genetic stability of the inserted DNA over multiple generations in cotton events GHB614 and LLCotton25 was demonstrated previously (EFSA, 2006c, 2009a). Integrity of the events was demonstrated in cotton GHB614 × LLCotton25[11] by Southern analyses in the fourth self-pollinating generation after crossing the parental lines. Therefore, there is no indication of rearrangements resulting from an interaction between the events.

4.1.3. Information on the expression of the inserts[12]

Plants were grown at three locations (four replicate blocks) under field conditions in 2012 in USA. The two-event stack cotton and the single events were grown with and without their intended herbicide treatments. The 2mEPSPS and PAT proteins in the two-event stack cotton and the two single events were quantified by enzyme-linked immunosorbent assay (ELISA). The proteins were quantified in leaf (four- to seven-leaf stage), root (four- to seven-leaf stage), pre-candle squares (two weeks after flowering), immature boll (two weeks after flowering) and fuzzy seed (maturity).

The values for 2mEPSPS and PAT in the two-event stack cotton were compared with the corresponding values in the single cotton events.[13]

Substantial changes in protein expression values are expected if interactions at the DNA and RNA level, such as gene silencing, occur. Only small changes, if any, in protein expression values were observed (see Table 4 for an example in seed). Taking this into account, as well as the inherent variability of plants, the observed small changes, if any, do not indicate the occurrence of interactions between the events in the two-event stack cotton.

[11] Dossier: Part I—Moens (2010a).
[12] Dossier: Part I—Section D3.
[13] Additional information, March 2013.

Table 4: Means, standard deviations and ranges of newly expressed protein levels in seed at the fuzzy seed stage (µg/g dry weight) from cotton GHB614, LLCotton25 and the two-event stack cotton

Event/ protein	With intended herbicides treatment			Without intended herbicides treatment		
	GHB614 × LLCotton25	GHB614	LLCotton25	GHB614 × LLCotton25	GHB614	LLCotton 25
2mEPSPS	97.1[a] ± 16[b]	93.0 ± 19	–	109.0 [(a)] ± 11 [(b)]	113.0 ± 13	–
	71.6–126[c]	53.6–123		93.3–127 [(c)]	53.6–123	
PAT	183 ± 22	–	178 ± 26	203 ± 27	–	204 ± 33
	153–224		131–234	135-233		150–248

(a): Mean.
(b): Standard deviation.
(c): Range.
–, Not assayed.

4.1.4. Conclusion of the molecular characterisation

Molecular characterisation did not identify issues requiring specific investigation.

4.2. Comparative analysis

4.2.1. Production of material for the comparative assessment

The application EFSA-GMO-NL-2010-77 presented compositional, agronomic and phenotypic characteristics data on cotton collected in a field trial performed in the USA in 2008.[14] The field trial included the stacked cotton GHB614 × LLCotton25, its conventional counterpart and the single cotton events LLCotton25 and GHB614. The conventional counterpart was the non-GM cotton variety FM 958 (*G. hirsutum*), which was used as the recurrent parent for breeding cotton GHB614 × LLCotton25.

The field trial was conducted during the 2008 growing season in seven locations in the south-eastern United States, representing typical cotton-growing regions. A randomised complete block design with three repetitions and five treatment regimens was used. The conventional counterpart, FM 958, received location-specific maintenance pesticide management only; cotton GHB614 × LLCotton25 received the maintenance management with and without additional application of -ammonium and glyphosate-based herbicides. The single cotton events LLCotton25 and GHB614, grown in the same field trials, received the respective intended herbicides on top of the maintenance pesticide management.

Comparative compositional analysis was performed on fuzzy cottonseed samples, which represent the starting material for all food and feed products produced from cotton.

4.2.2. Agronomic traits and GM phenotype

Measurements of agronomic characteristics included endpoints related to plant growth and morphology at different life stages, reproduction and fecundity, agricultural productivity, and disease susceptibility.[15]

Statistically significant differences were observed between cotton GHB614 × LLCotton25 and its conventional counterpart for the following endpoints: plant stand, number of days to first bloom, yield, per cent lint, fibre length, fibre elongation, boll size, seed index, number of nodes, first position bolls

[14] Dossier: Part I—Kowite (2009) and Oberdoerfer (2009).
[15] Plant stands or counts, strain uniformity, morphology ratings of leaves, flowers, bolls and plants, disease reactions, stalk lodging, days to first bloom, days to first open boll, boll type and size, per cent open bolls, lint yield, per cent lint, seeds per boll, seed index, fibre properties of length, length uniformity, strength, micronaire and elongation and plant mapping data of plant height, number of nodes, number of first position bolls and total bolls.

and total bolls (Table 5). None of these differences occurred consistently at each location except for per cent lint, which was lower in the double-event stack cotton at the majority of sites.

The values observed for per cent lint fell within the range usually observed in commercial non-GM cotton (e.g. Bourland et al., 2014; University of Georgia, 2014). The differences for all the parameters are of the same order of magnitude as those expected between different cotton lines. Furthermore, the EFSA GMO Panel considered that the magnitude of the differences in the specific parameters shown in Table 5 would not be of biological relevance for food/feed safety; therefore, they are not assessed further in Section 4.3. These statistically significant differences are further assessed for their potential environmental impact in Section 4.4.

Table 5: Agronomic and phenotypic endpoints for which a statistically significant difference was observed between the cotton GHB614 × LLCotton25 treated or non-treated with the intended herbicides and its conventional counterpart FM 958. The mean values together with their standard deviations are given.

Endpoint	**FM 958**	**Cotton GHB614 × LLCotton25**	
		Non-treated	**Intended herbicide treated**
Plant stand (plant/ft)	2.78 ± 0.56	2.90±0.61*	3.10 ± 0.62
First bloom (no of days)	52.8 ± 6.1	52.1 ± 5.9	52.1 ± 5.8
Yield (kg lint/acre)	431 ± 170	385 ± 131	406 ± 108*
Per cent lint (%)	40.5 ± 1.3	37.8 ± 2.6	38.1 ± 2.5
Boll size (g)	5.77 ± 0.58	6.0 ± 0.38	5.95 ± 0.41
Seed index (g)	10.54 ± 1.64	11.34 ± 1.22	11.14 ± 1.10
Fibre length (cm)	3.0 ± 0.1	3.0 ± 0.1*	3.1 ± 0.1
Fibre elongation (%)	4.6 ± 0.6	4.5 ± 0.5*	4.4 ± 0.5
Number of nodes	17.5 ± 1.8	16.6 ± 2.1	17.1 ± 1.6*
First position bolls (no/plant)	4.7 ± 1.2	4.1 ± 0.9	4.3 ± 0.8*
Total bolls (no/plant)	10.6 ± 2.2	8.6 ± 3.6	8.6 ± 1.9

*This value for cotton GHB614 × LLCotton25 is not statistically significant different, i.e. , no *p* value of less than 5 %, from that in FM 958.

4.2.3. Compositional analysis

Comparative compositional analysis was performed on fuzzy cottonseed samples obtained from cotton GHB614 × LLCotton25, the single cotton lines LLCotton25 and GHB614 and the conventional counterpart FM 958.

A total of 49 parameters, including proximates, minerals, amino acids, fatty acids and anti-nutrients/toxins, was analysed.[16] The selected compositional parameters are in line with recommendations laid down in the consensus document on key compositional parameters of cotton varieties published by the OECD Task Force on the Safety of Novel Foods and Feed (OECD, 2009).

Statistically significant differences in fuzzy cottonseed were observed for crude fat, ash, calcium, potassium, magnesium, iron, zinc, phytic acid, dihydrosterculic acid and free and total gossypol (Table 6). The levels of crude fat, ash, calcium, potassium, magnesium, zinc and dihydrosterculic acid observed in cotton GHB614 × LLCotton25 fell within the range of non-GM cotton seeds reported in literature (OECD, 2009; Calhoun et al., 1995, Lundquist, 1995). With the exception of magnesium,[17] these endpoints were also statistically significantly different between cotton GHB614 × LLCotton25 and its parental lines.

For the differences in the minerals potassium, magnesium, zinc and calcium, no further assessment was deemed necessary owing to their well-known biochemical roles and to the small absolute magnitude of the reported levels. For crude fat and ash, the magnitudes of the differences between the reported levels was low and the levels were within the levels reported in literature. The EFSA GMO Panel considered them of no biological relevance. Although the iron levels in cotton GHB614 × LLCotton25 in comparison with its conventional counterpart FM 958 were statistically significantly lower (max. 20 %), the EFSA GMO Panel noted that animal feed is usually supplemented with minerals to ensure wholesomeness, and cottonseed meal also specifically with iron salts to decrease the toxicity of gossypol, which is bound by iron. Cottonseed oil and linters are not a source of iron for human consumption; the contribution of iron for human consumption is restricted to flours from glandless cotton varieties that do not produce gossypol and can be considered negligible. The EFSA GMO Panel concludes that the difference in iron levels does not need further consideration.

The reduced concentration of phytic acid in cotton GHB614 × LLCotton25 was not considered to give rise to safety concerns, as it is an antinutrient. The small observed differences for dihydrosterculic acid are also not considered to be of concern, since the sum of all the cyclopropenic fatty acids in the same metabolic pathway (sterculic acid, dihydrosterculic and malvalic acid) did not differ. These parameters are therefore not assessed in the next section.

Taking also into consideration that gossypol is considered to be an undesirable substance in feed (EFSA, 2008),[18] further assessment of the observed difference in gossypol levels has been carried out in Section 4.3.

[16] Moisture, protein, total fat, ash, carbohydrates, acid detergent fibre (ADF), neutral detergent fibre (NDF), calcium, phosphorus, potassium, iron, magnesium, zinc, alpha-tocopherol, phytic acid, gossypol (free and total), sterculic acid, malvalic acid, dihydrosterculic acid, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine, tryptophan, myristic acid, palmitic acid, margaric acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, arachidic acid, behenic acid and lignoceric acid.

[17] Technical dossier D7.

[18] Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. OJ L 140, 30.5.2002, p. 10–22.

Table 6: Compositional endpoints in fuzzy cottonseeds harvested from field trials with cotton GHB614 × LLCotton25 treated or non-treated with the intended herbicides and its conventional counterpart FM 958 for which a statistically significant difference was observed (measured in dry matter). The mean values together with their standard deviations are given.

Parameter	FM 958	Cotton GHB614 × LLCotton25	
		Non-treated	Intended herbicide treated
Crude fat (%)	20.0 ± 1.6	20.2 ± 1.7*	20.7 ± 1.7
Ash (%)	4.04 ± 0.21	3.84 ± 0.19	3.84 ± 0.18
Calcium (%)	0.131 ± 0.02	0.125 ± 0.02	0.126 ± 0.02
Potassium (%)	1.23 ± 0.06	1.19 ± 0.06	1.19 ± 0.04
Magnesium (%)	0.37 ± 0.03	0.36 ± 0.02	0.37 ± 0.02*
Iron (mg/kg)	54.1 ± 13.3	46.5 ± 9.4	45.2 ± 7.3
Zinc (mg/kg)	34.9 ± 2.7	36.2 ± 3.4	34.8 ± 3.0*
Free gossypol (% DM)	0.54 ± 0.10	0.68 ± 0.12	0.70 ± 0.09
Total gossypol (% DM)	0.60 ± 0.09	0.74 ± 0.11	0.76 ± 0.09
Phytic acid (% DM)	1.77 ± 0.23	1.68 ± 0.21	1.68 ± 0.24
Dihydrosterculic acid (% rel.)	0.25 ± 0.03	0.26 ± 0.03	0.26 ± 0.03

DM, dry matter; % rel, per cent dihydrosterculic acid based on total amount of fatty acids.
*No statistically significant difference was identified.

4.2.4. Conclusion of the comparative assessment

The EFSA GMO Panel did not consider that the differences observed in the comparative assessment require further food and feed safety assessment except for the introduced genetically modified traits (2mEPSPS and PAT; Section 4.3) and gossypol (Section 4.3). The observed agronomic and phenotypic differences are assessed in Section 4.4.

4.3. Food/feed safety assessment

4.3.1. Effect of processing

The effect of processing on this cotton is not expected to be different from that of conventional cotton.

4.3.2. Toxicology

4.3.2.1. Assessment of newly expressed proteins in cotton GHB614 × LLCotton25

The two new proteins expressed in cotton GHB614 × LLCotton25 (2mEPSPS and PAT) were previously assessed, and no safety concerns were identified for humans and animals (EFSA, 2006c, 2009a). The EFSA GMO Panel is not aware of any new information that would change these conclusions. In an updated literature review,[19] no new data relevant for the safety assessment of the newly expressed proteins from cotton GHB614 and LLcotton25 were identified.

Upon request,[20] the applicant provided updated (2013) bioinformatics studies in which the amino acid sequences of the proteins 2mEPSPS and PAT were compared with those of known proteins. These studies confirmed the absence of relevant similarities between the newly expressed proteins and known toxins.

The levels of the newly expressed proteins in cotton GHB614 × LLCotton25 are comparable to those in the corresponding single cotton events (see Section 4.1).

[19] Additional information, March 2013.
[20] Additional information, December 2013.

A potential for adverse effects, or lack of adverse effects, may be predicted using generally accepted concepts of mixture toxicology. In particular, depending on the type of joined action, three major predictive models of mixture effects, designated "interaction", "dose addition" or "response addition", may be applied (Cassee et al., 1998; Groten et al., 2001).

i. "Interactions" take place only when single components of a mixture are able to influence the toxicity of one another, leading to a synergistic or antagonistic outcome in combination.

ii. If, however, the individual components of mixtures do not influence the toxicity of one another, they may act independently on a common biological target, in which case the "dose addition" model should be applied to predict their combined effect.

iii. If, finally, the components of mixtures act independently on distinctly different biological targets, the "response addition" model is applicable to predict their combined outcome.

The GMO Panel used these concepts of mixture toxicology to predict the potential for the combined action of the newly expressed proteins occurring in cotton GHB614 × LLCotton25. On the basis of the known biological properties of the individual newly expressed proteins, there is currently no expectation for possible interactions relevant for animal and human toxicity among them in cotton GHB614 × LLCotton25. The activities of the newly expressed enzymes 2mEPSPS and PAT involve distinctly different substrates, thus dismissing the possibility of a combined action following a "dose addition" model. These considerations led to the conclusion that the "response addition" model is best suited to estimate the safety of the newly expressed proteins in this two-event stack cotton. Given that no adverse effects in the available toxicological studies were observed, and since there were no structural similarities to known toxins, the "response addition model" predicts that the newly expressed proteins in the two-event stack cotton would not give rise to safety concerns for human and animal health.

4.3.2.2. Assessment of constituents other than proteins

This section focuses on the toxicological and nutritional assessment of elevated gossypol levels found in cotton GHB614 × LLCotton25, when compared to its conventional counterpart and both parental lines (see Section 4.1.2).

The EFSA Panel on Contaminants in the Food Chain described gossypol as undesirable substance in animal feed (EFSA, 2008). Current EU feed legislation has introduced limits to the maximum contents of free gossypol for feed materials and complete feeds.[21] These limits are not allowed to be exceeded in order to protect target species and consumers of animal products derived from animals fed gossypol containing feed.

Direct human exposure to cottonseed products is predominantly via the refined oil, which is essentially free from gossypol (0–0.09 % DM total gossypol). An alternative route of exposure may arise from the use of flour prepared from seeds. However, the source of such flours is gland-free varieties, which do not produce gossypol. The EFSA GMO Panel is of the opinion that the production of flour from GHB614 × LLCotton25 cottonseeds is suitable only if the event is introduced into a genetic background resulting in a gland-free cotton variety.

Having considered the gossypol content of cottonseed, the gossypol toxicity in animals and humans, the effect of processing on the gossypol content of cottonseed feed materials and the use of certain cottonseed materials as food or feed, the EFSA GMO Panel concludes that the higher content of gossypol in cottonseed of GHB614 × LLCotton25 (both treated and untreated with the intended herbicides) is of no safety concerns for animals and humans in practice because (i) the maximum content of free gossypol in feed, is regulated by European legislation;[21] and (ii) bleached and refined

[21] Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. OJ L 140, 30.5.2002, p. 10–22.

cottonseed oil as well as flour produced from cottonseed, which may be directly consumed by humans, is free from detectable traces of free gossypol.

4.3.3. Animal studies with food/feed derived from GM plant

In the present assessment, no change in the integrity of the inserts in cotton GHB614 × LLCotton25 was found when they were compared with the corresponding single events during the molecular characterisation. Furthermore, the levels of the newly expressed proteins in cotton GHB614 × LLCotton25 are comparable to those in the corresponding single cotton events (see Section 4.1). The increased levels of gossypol in the whole cottonseed from cotton GHB614 × LLCotton25 (see Sections 4.2.3 and 4.2.4) do not pose concerns for human or animal safety (see Section 4.3.2.2). Moreover, no biologically relevant differences in phenotypic and agronomic characteristics of cotton GHB614 × LLCotton25 were identified (see Sections 4.1.3 and 4.1.4). The EFSA GMO Panel considered all the data available and is of the opinion that interactions between the cotton events impacting the food and feed safety of cotton GHB614 × LLCotton25 are unlikely. Therefore, the EFSA GMO Panel does not consider additional animal safety studies with the whole GM food/feed necessary.

4.3.4. Allergenicity

For allergenicity assessment, a weight-of-evidence approach is followed, taking into account all of the information obtained on the newly expressed proteins with various test methods, since no single experimental method yields decisive evidence on allergenicity (EFSA, 2006a, 2010, 2011a; CAC, 2009). In addition, when known functional aspects of the newly expressed protein or structural similarity to known adjuvants may indicate an adjuvant activity, the possible role of these proteins as adjuvants is considered (EFSA, 2011a). When newly expressed proteins with a potential adjuvant activity are expressed together, possible interactions increasing adjuvanticity and impacting on the allergenicity of the GM crop are assessed.

4.3.4.1. Assessment of allergenicity of the newly expressed proteins

The EFSA GMO Panel has previously evaluated the safety of the 2mEPSPS and PAT proteins in the single events in the context of several other applications. No concerns on allergenicity were identified (e.g. EFSA, 2006c, 2009a). No new information on the single events that might change the previous conclusions of the EFSA GMO Panel has become available. No concerns regarding adjuvanticity of these newly expressed proteins have been identified in the scientific literature or in the bioinformatics analyses.

Interactions between the newly expressed proteins 2mEPSPS and PAT in cotton GHB614 × LLCotton25 impacting on their allergenicity and/or adjuvanticity are not expected given the lack of indications of allergenicity and adjuvanticity of the individual proteins.

4.3.4.2. Assessment of allergenicity of the GM plant

Cotton is not considered to be a common allergenic food (OECD, 2009).[22] A few cases of food allergy to cottonseed have been reported (Atkins, 1988; Malanin and Kalimo, 1988; O'Neil and Lehrer, 1989; de Olano et al., 2009; Mane et al., 2013), all of which were to foods with cottonseed flour as the offending ingredient. However, the main cottonseed product in human food, industrially processed cottonseed oil, is highly purified and contains negligible levels of proteins. Also, in cellulose from cottonseed linters for food use, the protein level is very low.

The EFSA GMO Panel identified no indications of safety concerns regarding the overall allergenicity of cotton GHB614 × LLCotton25.

[22] Directive 2007/68/EC of the European Parliament and of the Council of 27 November 2007 amending Annex IIIa to Directive 2000/13/EC of the European Parliament and of the Council as regards certain food ingredients. OJ, L310, 11–14.

4.3.5. Nutritional assessment of GM food/feed

The intended traits of cotton GHB614 × LLCotton25 are two types of herbicide tolerance, with no intention to alter the nutritional parameters. Compositional analysis indicated increased levels of gossypol in the whole cottonseed from cotton GHB614 × LLCotton25 as the only relevant change (see Sections 4.1.3 and 4.1.4), but this was considered not to pose a practical concern for human or animal safety (see Section 4.3.2.2). Based on the result of the compositional analysis, the EFSA GMO panel concludes that cottonseed of GHB614 × LLCotton25 is expected to be as nutritious as its conventional counterpart.

4.3.6. Conclusion

The newly expressed proteins in cotton GHB614 × LLCotton25 have been individually assessed previously and no safety concerns were identified. There are no new data, which would lead to a revision of these conclusions. Moreover, no interactions between the newly expressed proteins are expected based on their biological properties. The EFSA GMO Panel identified no indications of safety concerns regarding the overall allergenicity of cotton GHB614 × LLCotton25. The cottonseed of GHB614 × LLCotton25 is as nutritious as that of its conventional counterpart.

4.4. Environmental risk assessment and monitoring

4.4.1. Evaluation of relevant scientific data

Considering the scope of application EFSA-GMO-NL-2009-77, the environmental risk assessment of cotton GHB614 × LLCotton25 is concerned with (i) exposure of bacteria to recombinant DNA in the gastrointestinal tract of animals fed GM material and those present in environments exposed to faecal material; and (ii) accidental release into the environment of viable grains of cotton GHB614 × LLCotton25 during transportation and processing.

As the scope of the present application excludes cultivation, environmental concerns in the EU related to the use of glyphosate and glufosinate-ammonium-based herbicides on the GM cotton do not apply.

4.4.2. Evaluation of the single events GHB614 and LLCotton25

In its previous scientific opinions, the EFSA GMO Panel was of the opinion that both single cotton events GHB614 and LLCotton25 are as safe as their conventional counterparts and that the placing on the market of cotton GHB614 and LLCotton25, for import and processing for food and feed uses, is unlikely to have an adverse effect on human or animal health, or on the environment (EFSA, 2006, 2008). Furthermore, the post-market environmental monitoring (PMEM) plans for cotton GHB614 and LLCotton25 as proposed by the applicant were in line with EFSA GMO Panel opinion on PMEM of GM plants (EFSA, 2011b).

4.4.3. Environmental risk assessment

4.4.3.1. Unintended effects on plant fitness due to the genetic modification

Gossypium hirsutum is a highly domesticated crop which has been grown in southern Europe since the 19th century, giving rise to feral plants which can occasionally be found in the same area (Davies, 1967; Todaro, 1917). The main cultivated cotton species (*G. hirsutum*) is an annual self-pollinating crop. In the absence of insect pollinators (such as wild bees, honey bees, bumble bees), cotton flowers are self-pollinating, but when these pollinators are present low percentages of cross-pollination occur (McGregor, 1959; Moffett and Stith, 1972; Moffett et al., 1975; Van Deynze et al., 2005).

Pollen and cottonseed dispersal are potential sources of vertical gene flow to other cotton varieties, and to occasional feral cotton plants only, since in Europe there are no cross-compatible wild relatives. Because cotton pollen is very large (120–200 μm), heavy and sticky, wind-mediated dispersal of pollen to other cotton varieties is negligible (Vaissiere and Vinson, 1994). In addition, cross-pollination percentages rapidly decrease with increasing distance from the pollen source (Umbeck et

al., 1991; Kareiva et al., 1994; Llewellyn and Fitt, 1996; Xanthopoulos and Kechagia, 2000; Zhang et al., 2005; Van Deynze et al., 2005; Hofs et al., 2007; Llewellyn et al., 2007).

Seeds are the only survival structures. However, seed-mediated establishment of cotton and its survival outside cultivation in Europe is mainly limited by a combination of absence of a dormancy phase, low competitiveness and susceptibility to diseases and cold climate conditions (Eastick and Hearnden, 2006). In regions where cotton is widely grown, such as Australia, the risk of GM cotton becoming feral along transportation routes, or a weed on dairy farms where raw cottonseed is used as feed, has been shown to be negligible (Addison et al., 2007). Adequate soil moisture is an additional factor affecting the survival of feral cotton seedlings.

The relevance of the 11 parameters (see Table 5) for which significant differences were observed in the agronomic and phenotypic field trials discussed in Section 4.2.2 is assessed here. The EFSA GMO Panel considers that, in the context of the scope of this application, the magnitude of the differences observed is unlikely to affect significantly the overall fitness, invasiveness or weediness of the GHB614 × LLCotton25. Therefore, the accidental release of GHB614 × LLCotton25 seeds (i.e. during transport and/or processing) would not result in the establishment of plants exhibiting dissemination capabilities different from existing conventional cotton varieties and would not create additional agronomic or environmental impacts.

In addition to the data presented by the applicant, the EFSA GMO Panel is not aware of any scientific report of increased fecundity, persistence (volunteerism) or ferality of GM cotton in regions where it is cultivated (Bagavathiannan and Van Acker, 2008; Eastick and Hearnden, 2006). There is no information that indicates a change in survival capacity (including overwintering).

Furthermore, there is no evidence that the herbicide tolerance traits result in increased persistence and invasiveness of any crop species, except where glyphosate or glufosinate-ammonium herbicides are applied. Thus, escaped plants and genes dispersed to other cotton plants would result in plant populations no different from existing populations and would not create additional agronomic or environmental impacts.

Since the general characteristics of cotton GHB614 × LLCotton25 are unchanged relative to its conventional counterpart, the inserted herbicide tolerance traits are not likely to provide a selective advantage outside cultivation in Europe. If accidental spillage and subsequent release into the environment of cotton GHB614 × LLCotton25 seeds occur, cotton GHB614 × LLCotton25 plants would have a selective advantage only when and where glyphosate or glufosinate-ammonium herbicides are applied. These herbicides are not commonly used on cultivated cotton or in most areas in the EU where the GM cotton might be spilled. It is thus considered very unlikely that cotton GHB614 × LLCotton25, or its progeny, will differ from other cotton varieties in its ability to survive until subsequent seasons or to establish feral populations under European environmental conditions.

Cotton GHB614 × LLCotton25 will be imported as mostly non-viable seed; therefore, the likelihood that imported GM seeds will germinate and potentially establish a feral cotton population in the European environment can be considered negligible.

Consequently, the EFSA GMO Panel concluded that there is no indication of an increased persistence and invasiveness potential of cotton GHB614 × LLCotton25 compared with conventional cotton. It can therefore be considered that cotton GHB614 × LLCotton25 has no altered survival, multiplication or dissemination characteristics compared with its conventional counterpart or with its parental lines, except under application of the intended herbicides.

4.4.3.2. Gene transfer

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either horizontal gene transfer of DNA or vertical gene flow via cottonseed dispersal and cross-pollination.

(a) Plant to bacteria 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 digestion in the human or animal gastrointestinal tract. However, a low level of exposure of fragments of ingested DNA, including the recombinant fraction of such DNA, to bacteria in the digestive tract of humans, domesticated animals and other animals feeding on cotton GHB614 × LLCotton25 is expected.

Current scientific knowledge of recombination processes in bacteria indicates that horizontal transfer of non-mobile, chromosomally located DNA fragments between unrelated organisms (such as plants to microorganisms) is not expected to occur at detectable frequencies under natural conditions (see EFSA, 2009b, for further details).

A successful horizontal transfer would require stable insertion of the transgene sequences into a bacterial genome and a selective advantage conferred on the transformed host. The only known mechanism that facilitates horizontal transfer of non-mobile, chromosomal DNA fragments into bacterial genomes is homologous recombination. This requires the presence of stretches of DNA sequences that are similar in the recombining DNA molecules and, in addition to substitutive gene replacement, facilitates the insertion of non-homologous DNA sequences if their flanking regions share sequence similarity with bacterial sequences in the recipient.

Cotton GHB614 × LLCotton25 contains one genetic element sharing homology to those in bacteria, i.e. the coding sequence of the phosphinothricin acetyltransferase (*bar*) gene of *Streptomyces hygroscopicus*. Homologous recombination of the *bar* gene with natural variants of this gene as they may occur in *S. hygroscopicus* and other Actinobacteria would only replace natural sequences and not provide any new property to the bacteria. The coding sequence of the 2m*epsps* gene originates from maize. The flanking regions of the two recombinant genes provide no DNA homology, which would facilitate horizontal gene transfer to the Ti-plasmid of *Agrobacterium tumefaciens*. *S. hygroscopicus* can be isolated from soil and is not considered to be prevalent in the main receiving environment, i.e. the gastrointestinal tract of humans or animals. 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. 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 expected to be 10^{10}-fold lower than for homologous recombination (Hülter and Wackernagel, 2008; EFSA, 2009b).

The *bar* gene of cotton GHB614 × LLCotton25 is regulated by the 35S promoter of the cauliflower mosaic virus and the *2*m*epsps* gene is regulated by a eukaryotic plant promoter (derived from the *Arabidopsis thaliana* histone H4 gene). The expression of these constructs in bacteria is unknown, but generally the expression level of eukaryotic promoters in bacteria is inefficient (Warren et al., 2008).

The EFSA GMO Panel concludes that there is no indication for horizontal gene transfer from the 2m*epsps* maize gene to bacteria. Moreover, it concludes that a horizontal transfer of the *bar* gene from cotton GHB614 × LLCotton25 to bacteria would replace only natural variants (i.e. substitutive recombination) of such genes and therefore it is unlikely to provide any new property connected to a selective advantage for the recipient organisms. Considering its intended use as food and feed and the above assessment, the EFSA GMO Panel has therefore not identified any concern associated with horizontal gene transfer from cotton GHB614 × LLCotton25 to bacteria.

(b) Plant to plant gene transfer

Considering the intended uses of cotton GHB614 × LLCotton25 and the physical characteristics of cottonseed, a possible pathway of dispersal is from cottonseed spillage and pollen of occasional feral

GM cotton plants originating from accidental cottonseed spillage during transportation and/or processing.

The genus *Gossypium* consists of at least four crop species: *Gossypium arboreum*, *Gossypium barbadense*, *Gossypium herbaceum* and *Gossypium hirsutum*. *G. herbaceum* is reported (Zohary and Hopf, 2000) to have been a traditional fibre crop in the eastern Mediterranean area even in the pre-Columbus period (before 1500 AD). In southern Europe, *G. herbaceum* and *G. hirsutum* have been grown since the 19th century, giving rise to occasional feral plants in the same area (Todaro, 1917; Davies, 1967; Zangheri, 1976; Tutin et al., 1992) but no sexually compatible wild relatives of *G. hirsutum* have been reported in Europe. Therefore, the plant to plant gene transfer from this GM cotton is restricted to cultivated and occasional feral cotton populations. The EFSA GMO Panel also takes into account the fact that this application does not include cultivation of the GM cotton within the EU so that the likelihood of cross-pollination between the imported GM cotton and cotton crops and occasional feral cotton plants is considered to be extremely low. Even if feral populations of cotton GHB614 × LLCotton25 were to be established or transgene flow occurred to cultivated and feral cotton, a selective advantage would occur only in the presence of glyphosate or glufosinate-ammonium herbicides, which are not commonly used in most areas where the GM cotton might be spilled.

4.4.3.3. Interactions of the GM plant with target organisms

Considering the scope of the application, excluding cultivation, and the absence of target organisms, potential interactions of the GM plant with target organisms were not considered a relevant issue by the EFSA GMO Panel.

4.4.3.4. Interactions of the GM plant with non-target organisms

Considering the scope of the application, excluding cultivation, and owing to the low level of exposure to the environment, potential interactions of the GM plant with non-target organisms were not considered a relevant issue by the EFSA GMO Panel.

4.4.3.5. Interactions with the abiotic environment and biogeochemical cycles

Considering the scope of the application, excluding cultivation, and owing to the low level of exposure to the environment, potential interactions of the GM plant with the abiotic environment and biogeochemical cycles were not considered a relevant issue by the EFSA GMO Panel.

4.4.4. Conclusion

If accidental spillage and subsequent release into the environment of cotton GHB614 × LLCotton25 seeds occur, cotton GHB614 × LLCotton25 plants would have a selective advantage only in the presence of glyphosate or glufosinate-ammonium herbicides which are not commonly used on cultivated cotton or in most areas where the GM cotton might be spilled. In addition, the low levels of environmental exposure of these GM cotton plants and the newly expressed proteins through other routes indicate that the risk to non-target organisms is extremely low. The EFSA GMO Panel considers unlikely that the recombinant DNA in cotton GHB614 × LLCotton25 would transfer to bacteria. The risk caused by a rare but theoretically possible transfer of the recombinant genes from cotton GHB614 × LLCotton25 to bacteria is regarded as negligible owing to the lack of a selective advantage in the context of its intended uses. The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of cotton GHB614 × LLCotton25.

The EFSA GMO Panel is aware that, owing to physical characteristics of cotton seed and methods of transportation, accidental spillage cannot be excluded. Therefore, the EFSA GMO Panel recommends that appropriate management systems are introduced to actively monitor the occurrence of feral cotton plants in areas where spillage and cotton plant establishment are likely to occur. The EFSA GMO Panel also recommends that appropriate management systems should be in place to restrict seeds of

cotton GHB614 × LLCotton25 from entering cultivation as this would require specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

5. POST-MARKET MONITORING

5.1. Post-market monitoring of GM food/feed

No data indicating that cotton GHB614 × LLCotton25 is any less safe than its conventional counterpart have emerged. In addition, cotton GHB614 × LLCotton25 is as nutritious as its conventional counterpart. Therefore, and in line with the Guidance Documents (EFSA, 2006a, 2011a), the EFSA GMO Panel is of the opinion that post-market monitoring of the GM food/feed is not necessary.

5.2. Post-market environment monitoring

The objectives of a monitoring plan according to Annex VII of Directive 2001/18/EC are to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the environmental risk assessment are correct and to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment which were not anticipated in the environmental risk assessment.

Monitoring is also related to risk management, and thus a final adoption of the PMEM plan falls outside the mandate of EFSA. However, the EFSA GMO Panel gives its opinion on the scientific content of the monitoring plan provided by the applicant (EFSA, 2006a, b). The only significant exposure of the environment to the genetically modified cotton would be through faecal material or through accidental release into the environment of GM cotton grains during transportation and processing. Cotton GHB614 × LLCotton25 will be imported primarily as non-viable seed. Therefore, the likelihood that some imported seed escaping from silos or lorries could germinate is very low. The EFSA GMO Panel is aware that, owing to the physical characteristics of cottonseed and methods of transportation, accidental spillage cannot be excluded. Therefore, the EFSA GMO Panel recommends that appropriate management systems are introduced to actively monitor the occurrence of feral cotton plants in areas where cottonseed spillage and plant establishment are likely to occur.

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

The EFSA GMO Panel is of the opinion that the scope of the PMEM plan proposed by the applicant is in line with the intended uses of cotton GHB614 × LLCotton25 since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. No case-specific monitoring is necessary. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

CONCLUSIONS AND RECOMMENDATIONS

No new data leading to a modification of the previous conclusions on the safety of the two cotton events GHB614 and LLCotton25, the two parental lines of GHB614 × LLCotton25, was identified.

The combination of cotton events GHB614 and LLCotton25 in the two-event stack cotton did not raise molecular characterisation issues requiring further investigation. Except for the higher levels of gossypol in GHB614 × LLCotton25 compared with its conventional counterpart, the two-event stack cotton does not show any compositional or agronomic and phenotypic difference from its conventional counterpart that would require further food and feed safety assessment. The EFSA GMO Panel

concluded that the increased levels of gossypol in the whole cottonseed from cotton GHB614 × LLCotton25 do not pose concerns for human or animal safety. The newly expressed proteins in the two-event stack cotton do not raise safety concerns for human and animal health, and no interactions between these proteins are expected. Cotton GHB614 × LLCotton25 is expected to be as safe and nutritious as conventional cotton.

If accidental spillage and subsequent release into the environment of cotton GHB614 × LLCotton25 seeds occur, cotton GHB614 × LLCotton25 plants would have a selective advantage only in the presence of glyphosate or glufosinate-ammonium herbicides, which are not commonly used on cultivated cotton or in most areas where the GM cotton might be spilled. In addition, the low levels of environmental exposure of these GM cotton plants and the newly expressed proteins through other routes indicate that the risk to non-target organisms is extremely low. The EFSA GMO Panel considers it unlikely that the recombinant DNA in cotton GHB614 × LLCotton25 would transfer to bacteria. The risk caused by a rare but theoretically possible transfer of the recombinant genes from cotton GHB614 × LLCotton25 to bacteria is regarded as negligible owing to the lack of a selective advantage in the context of its intended uses. The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses. The EFSA GMO Panel recommends that appropriate management systems are introduced to actively monitor the occurrence of feral cotton plants in areas where spillage and cotton plant establishment are likely to occur. The EFSA GMO Panel also recommends that appropriate management systems should be in place to restrict seeds of cotton GHB614 × LLCotton25 entering cultivation as this would require specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

In conclusion, the EFSA GMO Panel considers that the information available for cotton GHB614 × LLCotton25 addresses the scientific comments raised by Member States. The EFSA GMO Panel is of the opinion that crossing cotton GHB614 and cotton LLCotton25 to produce cotton GHB614 × LLCotton25 results in no interactions affecting the safety of cotton GHB614 × LLCotton25 with respect to potential effects on human and animal health, or on the environment, in the context of its intended uses. The EFSA GMO Panel concludes that cotton GHB614 × LLCotton25 is as safe as its conventional counterpart and commercial cotton varieties and that it is unlikely to have any adverse effect on human and animal health, or on the environment, in the context of its intended uses.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of the Netherlands received on 4 February 2010 concerning a request for placing on the market of cotton GHB614 × LLCotton25 in accordance with Regulation (EC) No 1829/2003.

2. Acknowledgement letter dated 16 February 2010 from EFSA to the Competent Authority of the Netherlands.

3. Letter from EFSA to the applicant dated 17 March 2010 requesting additional information under completeness check.

4. Letter from the applicant to EFSA received 2 August 2010 providing additional information requested by EFSA under completeness check.

5. Letter from EFSA to the applicant dated 23 August 2010 requesting additional information under completeness check.

6. Letter from the applicant to EFSA received 11 November 2010 providing additional information requested by EFSA under completeness check.

7. Letter from EFSA to the applicant dated 6 December 2010 requesting additional information under completeness check.

8. Letter from the applicant to EFSA received 3 January 2011 providing the additional information requested by EFSA under completeness check.

9. Letter from EFSA to the applicant dated 26 January 2011, delivering the 'Statement of Validity' for application EFSA-GMO-NL-2010-77, cotton GHB614 × LLCotton25 submitted by Bayer under Regulation (EC) No 1829/2003.

10. Letter from EFSA to applicant dated 15 April 2011 requesting additional information and stopping the clock.

11. Letter from applicant to EFSA received 13 May 2011 providing additional information.

12. Letter from EFSA to applicant dated 16 September 2011 requesting additional information and maintaining the clock stopped.

13. Letter from applicant to EFSA received 18 March 2013 providing the additional information requested.

14. Letter from EFSA to applicant dated 3 October 2013 re-starting the clock.

15. Letter from EFSA to applicant dated 25 November 2013 requesting additional information and stopping clock.

16. Letter from the applicant to EFSA received 23 December 2013 providing additional information.

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