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SCIENTIFIC OPINION

Application (Reference EFSA-GMO-NL-2008-51) for the placing on the market of glyphosate tolerant genetically modified cotton GHB614, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience[1]

Scientific Opinion of the Panel on Genetically Modified Organisms

(Question No EFSA-Q-2008-016)

Adopted on 05 March 2009

PANEL MEMBERS*

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SUMMARY

This document provides a scientific opinion of the Scientific Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority (EFSA) on genetically modified (GM) cotton GHB614 (Unique Identifier BCS-GHØØ2-5) developed to provide tolerance to glyphosate-based herbicides.

In delivering its scientific opinion, the EFSA GMO Panel considered the application EFSA-GMO-NL-2008-51, additional information supplied by the applicant and scientific comments submitted by Member States. The scope of application EFSA-GMO-NL-2008-51 is for food and feed uses, import and processing of cotton GHB614 and all derived products, but excludes cultivation in the EU. The EFSA GMO Panel assessed cotton GHB614 with reference to the intended uses and appropriate principles described in the guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. The scientific assessment included molecular characterisation of the inserted DNA and expression of target proteins. A

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[*] (minority opinion) This opinion is not shared by 0 members of the Panel. / (conflict of interest) 0 members of the Panel did not participate in (part of) the discussion on the subject referred to above because of possible conflicts of interest.

comparative analysis of agronomic traits and composition was undertaken, and the safety of the new protein and the whole food/feed were evaluated with respect to potential toxicity, allergenicity and nutritional quality. An assessment of environmental impacts and the post-market environmental monitoring plan were undertaken.

Cotton GHB614 is derived from the cotton variety Coker 312 that was transformed by *Agrobacterium*-mediated gene transfer technology. Cotton GHB614 expresses a modified *epsps* (*2mepsps*) maize gene leading to the production of a modified 5-enolpyruvyl-shikimate-3-phosphate synthase (2mEPSPS) enzyme that confers tolerance to glyphosate-based herbicides.

The molecular characterisation data established that a single insert with one copy of the intact modified *epsps* (*2mepsps*) expression cassette is integrated in the cotton genomic DNA. Appropriate analyses of the integration site including sequence determination of the inserted DNA and flanking regions and bioinformatics analysis have been performed. Bioinformatics analysis of junction regions demonstrated the absence of any potential new open reading frames coding for known toxins or allergens. The expression of the gene introduced by genetic modification has been sufficiently analysed and the stability of the genetic modification has been demonstrated over several generations. The EFSA GMO Panel is of the opinion that the molecular characterisation of the DNA insert and flanking regions of cotton GHB614 does not raise any safety concern, and that sufficient evidence for the stability of the genetic modification was provided.

Based on comparative analyses, the EFSA GMO Panel concluded that cotton GHB614 is compositionally and agronomically equivalent to the non-GM counterpart and other conventional cotton except for the introduced trait. The risk assessment included an analysis of data from analytical studies, bioinformatics, and *in vitro* and *in vivo* studies. The EFSA GMO Panel considers it unlikely that the overall allergenicity of the whole plant is changed by the genetic modification and concludes that cotton GHB614 is as safe as the non-GM counterpart and other conventional cotton.

The application EFSA-GMO-NL-2008-51 concerns food and feed uses, import and processing. Therefore, there is no requirement for scientific information on possible environmental effects associated with the cultivation of cotton GHB614. The EFSA GMO Panel agrees that unintended environmental effects due to the establishment and spread of cotton GHB614 will not be different from that of conventionally bred cotton.

Considering the intended uses of cotton GHB614, the monitoring plan provided by the applicant is in line with both the EFSA GMO Panel guidance document on the risk assessment of GM plants and the opinion of the EFSA GMO Panel on post-market environmental monitoring. However, the EFSA GMO Panel is aware that, due to physical characteristics of cotton seeds and methods of transportation, accidental spillage is unavoidable. Therefore, the EFSA GMO Panel recommends that specific measures are introduced to actively monitor the occurrence of feral cotton plants in areas where seed spillage is likely to occur.

In conclusion, the EFSA GMO Panel considers that the information available for cotton GHB614 addresses the scientific comments raised by Member States and that cotton GHB614 is as safe as its conventional counterpart with respect to potential effects on human and animal health and the environment. The EFSA GMO Panel thus concludes that cotton

GHB614 is unlikely to have any adverse effect on human and animal health or on the environment in the context of its intended uses.

Key words: **GMOs, cotton, GHB614, BCS-GHØØ2-5, glyphosate tolerant, 2mEPSPS, food/feed safety, animal and human health, environment, import, processing, Regulation (EC) No 1829/2003**

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BACKGROUND

On 25 January 2008, the European Food Safety Authority (EFSA) received from the Netherlands an application (Reference EFSA-GMO-NL-2008-51), for authorisation of cotton GHB614 (Unique Identifier BCS-GHØØ2-5), submitted by Bayer CropScience within the framework of Regulation (EC) No 1829/2003 on genetically modified (GM) food and feed. After receiving the application EFSA-GMO-NL-2008-51 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 dossier available to the public 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 Regulation (EC) No 1829/2003. On 11 March 2008, EFSA declared the application as formally 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 following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Member State bodies had three months after the date of receipt of the valid application (until 11 June 2008) within which to make their opinion known.

The Scientific Panel on Genetically Modified Organisms (GMO Panel) of EFSA carried out a scientific assessment of the GM cotton GHB614 for food and feed uses, import and processing, in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003, taking into consideration the scientific comments of Member States and the additional information provided by the applicant (requested on 3 September 2008 and 12 November 2008).

In giving its scientific opinion on cotton GHB614 to the European Commission, 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 receipt 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.

TERMS OF REFERENCE

The EFSA GMO Panel was requested to carry out a scientific risk assessment of cotton GHB614 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 a scientific opinion on information required under Annex II to the Cartagena Protocol. Furthermore, the EFSA GMO Panel did also 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.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Groups on Molecular Characterisation, Food/Feed and Environment, as well as the following members of its staff: Yann Devos, Ana Gomes and Antonio Fernandez Dumont for the preparation of this opinion.

ASSESSMENT

1. Introduction

Cotton GHB614 (Unique Identifier BCS-GHØØ2-5) is assessed with reference to its intended uses and the appropriate principles described in the guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of GM plants and derived food and feed (EFSA, 2006a).

Cotton (*Gossypium hirsutum* L.) varieties derived from the GHB614 event express a modified 5-enopyruvyl-shikimate-3-phosphate synthase (2mEPSPS) of maize origin that is insensitive to broad-spectrum, post-emergent, foliar applied herbicides containing the active ingredient glyphosate.

2. Molecular characterisation

2.1. Issues raised by Member States

Issues raised by Member States are addressed in Annex G of the EFSA overall opinion.

2.2. Background data

2.2.1. Transformation process and vector constructs

Cotton tissue from variety Coker 312 was transformed by *Agrobacterium tumefaciens* using the binary vector system. The disarmed *A. tumefaciens* strain harboured the transformation vector pTEM2. This vector contained the T-DNA region, with the left and right borders (LB and RB) delimiting a single gene cassette for expression of the modified *epsps* gene, named *2mepsps*. This gene of maize origin codes for an EPSPS protein with two amino acid substitutions conferring insensitivity to glyphosate. The amino acid substitutions in 2mEPSPS are the same as in the modified EPSPS in the previously assessed event maize GA21 (EFSA, 2007a). *2mepsps* transcription is driven by the *Ph4a748At* (histone H4) gene promoter originating from *Arabidopsis thaliana*. High level constitutive expression is expected, especially in meristematic (rapidly growing) green tissues. The promoter is followed by the first intron of gene II of the histone H3.III variant of *A. thaliana* and by an optimized transit peptide (constructed from *Zea mays* and *Helianthus annuus* DNA sequences). Termination of transcription uses the 3' untranslated region of the histone H4 gene of *A. thaliana*.

The vector backbone, i.e. the sequences of pTEM2 located outside of the T-DNA and which are not aimed at integration, contains replication origins for plasmid maintenance in both *Escherichia coli* (ORI ColE1) and *A. tumefaciens* (ORI pVS1), a selectable marker gene conferring resistance to streptomycin and spectinomycin (*aadA*) for propagation and selection of the plasmid in *E. coli* and *A. tumefaciens*, a DNA region consisting of a fragment of the neomycin phosphotransferase coding sequence of the *npt*I gene from transposon Tn903 and residual sequences of *A. tumefaciens* origin (plasmid pTiAch5 flanking the left and right borders).

2.2.2. Transgenic constructs in the genetically modified plant

The DNA sequences actually inserted in the GHB614 event were characterized by Southern analysis and by PCR amplification of both the insert and the flanking regions.

The number of T-DNA copies was determined by Southern hybridization using a combination of 9 restriction enzymes and 5 probes corresponding to the full length T-DNA and to four internal fragments corresponding to the different components of the transgene cassette. The data demonstrate the presence of a single T-DNA insert, as well as its integrity as compared with the original transgene cassette in vector pTEM2.

PCR amplification of the single inserted T-DNA allowed sequence determination of the entire 3978 bp insert and established a perfect match with the corresponding sequence in the vector pTEM2. The 5' and 3' flanking sequences (738 bp and 214 bp respectively) were also PCR amplified and sequenced. Characterization of the wild type target locus was achieved by amplifying a 994 bp fragment from wild type cotton using primers derived from the 5' and 3' flanks of the T-DNA. Sequence alignment between the pre-insertion locus (from wild type cotton) and the insertion locus (from event GHB614) identified a 17 bp deletion at the junction between the T-DNA and genomic DNA.

Examination of the gene insertion site was performed by searching nucleotide sequence databases with the pre-insertion locus (947 bp) as query sequence (blastn algorithm). No similarity with known functional genes in plants or other organisms could be identified. Bioinformatic tools for the prediction of functional genes were used for analyzing the pre-insertion locus and a hypothetical protein coding gene preceded by putative promoter elements could be found on the reverse strand of the 5' flanking region. Protein database searches (blastx) identified several conserved polypeptides in plants, but with no know function. It seems likely that the T-DNA of cotton GHB614 was inserted near a protein coding gene of unknown function. However, there are no indications from comparative agronomic performance and compositional analyses of any unintended effect caused by the insertion.

The absence of vector backbone sequences in the GHB614 event was studied by Southern analysis, using overlapping probes covering the entire vector DNA. The absence of hybridization signals, with the appropriate controls, indicated that no vector sequence was integrated into the plant genome besides the T-DNA. Sequence analysis of the T-DNA insert and of its flanking regions in the plant confirmed that no vector sequence out of the T-DNA region was present in the transgene locus.

2.2.3. Information on the expression of the insert

2.2.3.1. Expression of the introduced gene

2mepsps is the only gene potentially expressed from the transgene cassette in event GHB614.

The leaves of the cotton plant are the principal organs exposed to herbicide applications and commercial-level herbicide tolerance depends upon the function of the 2mEPSPS enzyme in the leaves. As a constitutive promoter with high activity in the leaves and meristematic tissues, the *Arabidopsis* histone H4 promoter was chosen to drive the expression of the *2mepsps* gene.

Expression level (data provided on a fresh weight basis) was measured by 2mEPSPS protein specific ELISA. Tissue samples were harvested from greenhouse grown cotton, at the 2-3 and 4-6 leaf stages of growth, pre-flowering and at flowering. It was found that 2mEPSPS protein ranged between 0.45 - 11.16 µg/g of leaves, 0.99 - 4.04 µg/g of roots, 1.58 - 1.94 µg/g of stems, depending on the growth stage of the plant, and was 5.47 ± 0.22 µg/g of apices, 5.35 ± 0.25 µg/g of squares (flower buds) and 0.16 ± 0.01 µg/g of pollen. Expressed as a percentage of total extractable protein, the 2mEPSPS protein showed a maximum of 0.39 % in leaves, 0.34 % in apices, 0.18 % in roots and squares, 0.06 % in stems and 0.001 % in pollen of cotton event GHB614. From published experience with the promoter and intron used, GHB614 plants were expected to show high levels of 2mEPSPS protein in rapidly growing plant parts, and lesser amounts in the other organs. Indeed, the following order of 2mEPSPS expression was found: leaf, apex >> roots, squares >> stems, seeds >> pollen.

The 2mEPSPS protein was also tested in seeds and processed seed fractions from unsprayed and sprayed plants produced in field trials in the US. The average 2mEPSPS protein content per test site in the field trial ranged from 15.8 µg/g to 25.5 µg/g in unsprayed fuzzy seed (overall average value of 19.2 ± 3.1 µg/g) and from 16.2 µg/g to 30.5 µg/g in sprayed fuzzy seed (overall average value of 21.2 ± 4.0 µg/g). The amount of 2mEPSPS protein was measured in 9 fractions of cottonseed, only 3 fractions contained detectable amounts of 2mEPSPS protein (delinted cottonseed: 102 ± 2 µg/g; hulls: 6.93 ± 0.40 µg/g; defatted meal: 0.26 ± 0.10 µg/g); the other fractions contained 6.63 µg/g.

2.2.3.2. Putative cryptic open reading frames

Open reading frame (ORF) and gene search tools were applied to predict the presence of potential newly created coding sequences both in the 5-prime flanking genomic/insert DNA junction region and in the insert/3-prime flanking genomic DNA junction region. Fourteen newly created ORFs were found that span the 5-prime and 3-prime junctions. In the unlikely event that the putative ORFs would be translated, bioinformatics analysis indicated that their putative translation products have no homology with any known toxins or allergens.

2.2.4. Information and stability of inserted DNA

The trait is inherited as a single dominant gene. Stability of the inserted DNA was demonstrated by Southern blot analysis of plants of multiple generations (from self-crosses and backcrosses into two genetic backgrounds), from different locations and environmental growth conditions. All tested samples showed the expected restriction enzyme digestion products.

Phenotypic stability was demonstrated by Mendelian inheritance of the herbicide tolerance trait over multiple generations and field locations, as well as throughout the development of commercial lines based upon cotton event GHB614.

2.3. Conclusion

The molecular characterisation data establish that the genetically modified cotton GHB614 contains one copy of an intact expression cassette with a modified maize *epsps* gene. No other parts of the plasmid used for transformation are present in the transformed plant. Bioinformatics analysis of the 5' and 3' flanking regions did not reveal any putative peptides

that would cause safety concerns. The stability of the inserted DNA and the herbicide tolerance trait were confirmed over several generations and a Mendelian inheritance pattern demonstrated.

3. Comparative analysis

3.1. Issues raised by Member States

Issues raised by Member States are addressed in Annex G of the EFSA overall opinion.

3.2. Evaluation of relevant scientific data

Having considered the information provided in the application and the Member States comments, the EFSA GMO Panel requested from the applicant further information with respect to the identity and breeding scheme of the non-GM comparator used in the agronomic / compositional analysis. The EFSA GMO Panel asked the applicant to check the consistency of some agronomic data presented in the application. The applicant provided the additional information as well as corrected agronomic data that the EFSA GMO Panel found adequate.

3.2.1. Choice of comparator and production of material for the compositional assessment

For compositional studies, cotton GHB614 was compared to its parent variety Coker 312. Data from the scientific literature regarding the natural ranges of key compounds in conventional cotton were also considered in the comparative assessment. Field trials with cotton GHB614 and its non-GM comparator Coker 312 were performed in the major cotton growing regions of the US in 2005 (9 sites) and 2006 (8 sites). In the year 2006, 8 trials were conducted at the same locations used the year before. Trials comprised 3 treatments at each location and 3 replications per treatment. The 3 treatments consisted of: (a) non-GM cotton grown using conventional herbicide weed control, (b) GM cotton grown using conventional herbicide weed control, and (c) GM cotton grown with glyphosate-based herbicide weed control.

3.2.2. Compositional analysis

Whole, linted cottonseed was used as suitable raw agricultural commodity for comparative compositional analysis. The seeds were analysed for key nutrients, anti-nutrients, and toxicants as defined by the OECD consensus document for cotton (OECD, 2004). Thus besides proximates (moisture, total fat, total protein, ash, total carbohydrates), acid detergent fibre (ADF), and neutral detergent fibre (NDF), the samples were analysed for 18 amino acids, 10 fatty acids (C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:0, C22:0, and C24:0), minerals (calcium, phosphorus, magnesium, potassium, iron, zinc), vitamin E, anti-nutrients (cyclopropenoid fatty acids and phytic acid) and the toxicant gossypol (free and total gossypol).

The statistical analysis of compositional data collected each year was carried out on a per location basis, using data from 3 replicates per location, and on the combined data from all sites each year. For most constituents, compositional differences between GHB614 cotton and its non-GM comparator occurred occasionally but not consistently over years and locations.

No change of the total amino acid composition was caused by the newly expressed protein-in GHB614 cotton.

For C16:1 (palmitoleic acid), C18:0 (stearic acid), C18:1 (oleic acid), C18:2 (linoleic acid) and C18:3 (linolenic acid) compositional differences were observed at 8, 11, 13, 12 and 17 out of the 17 field trial locations. However, differences were very small and are therefore not considered biologically relevant.

In case of the anti-nutritional cyclopropenoid fatty acids (CPFAs), the t-tests at the majority of per-location analyses found significantly lower values for sprayed and unsprayed cotton GHB614 versus the non-GM control. The estimated differences between the CPFAs mean values for the control and the GHB614 groups were all very small and are therefore not considered biologically relevant. There were no differences in the levels of free and bound gossypol.

All constituent levels for cotton GHB614 and the non-GM control fell inside the ranges of natural variability as reported in literature.

Besides the raw agricultural commodity, the chemical compositions of cottonseed linters, hulls, delinted seeds, meal, toasted meal, crude oil and refined, deodorised oil produced from cotton GHB614 and the non-GM counterpart harvested from one field trial were compared, and the analytical results assessed in light of the reference ranges in plant constituents reported in the literature. No nutritionally relevant differences were found. The obtained results support the conclusion with regard to compositional equivalence drawn for the raw agricultural commodity. No gossypol was detected in refined cottonseed oil obtained from cotton GHB614. The tendency of a slightly decreased content of CPFAs in cotton GHB614– as observed for whole linted cottonseed – was confirmed for the crude and refined oil.

The EFSA GMO Panel considered the observed compositional differences between cotton GHB614 and its non-GM comparator in the light of the field trial design and the natural ranges of the studied compounds reported for conventional cotton varieties. The EFSA GMO Panel concluded that cotton GHB614 (treated and untreated with the target herbicide) is compositionally equivalent to the non-GM counterpart and other conventional cotton, except for the introduced trait.

3.2.3. Agronomic traits and GM phenotype

The applicant provided information on agronomic performance and phenotypic characteristics derived from several field trials in the US performed in 2004 and 2005. Treatments consisted of: (a) non-GM cotton grown using conventional herbicide weed control, (b) GM cotton grown using conventional herbicide weed control, and (c) GM cotton grown with glyphosate-based herbicide weed control. The characteristics that were analysed in these studies included parameters related to plant morphology, seed and plant development, reproductive traits, disease and pest susceptibility, weediness, weed control, volunteers, yield, cotton seed and fibre quality.

The EFSA GMO Panel noted that differences were observed in some instances with regard to several characteristics related to yield, lint percentage, and reproduction. However, these differences did not occur consistently in the various studies and, therefore, were not considered to be related to the genetic modification.

The EFSA GMO Panel concludes that cotton GHB614 (treated and untreated with the target herbicide) is not agronomically different from other currently grown non-GM cotton varieties, with the exception of the newly introduced trait.

3.3. Conclusion

Compositional and agronomic analyses carried out on both glyphosate-treated and conventionally treated cotton GHB614, its non-GM comparator Coker 312 and other conventional cotton varieties treated with conventional herbicides indicated that cotton GHB614 is compositionally and agronomically equivalent to the non-GM counterpart and other conventional cotton, except for the introduced trait. The comparative analysis of cotton GHB614 therefore provided no indication for unintended effects resulting from the genetic modification.

4. Food/Feed safety assessment

4.1. Issues raised by Member States

Issues raised by Member States are addressed in Annex G of the EFSA overall opinion.

4.2. Evaluation of relevant scientific data

The EFSA GMO Panel has considered the information provided in the application and requested from the applicant further information with regards to the results of an acute oral toxicity study in mice using the 2mEPSPS protein, as well as the statistical analysis of data obtained in a broiler feeding study with seeds from cotton GHB614 and the identity of the non-GM comparator used in this study. Additional bioinformatics studies on potential homology of the 2mEPSPS protein to known toxic and allergenic proteins using up-to-date databases were also requested. The requested information was provided.

4.2.1. Product description and intended use

The scope of application EFSA-GMO-NL-2008-51 includes the import and processing of cotton GHB614 and its derived products for use as food and feed. Thus, the possible uses of cotton GHB614 include the production of refined oil from seeds and cellulose from linters for use as food or food ingredient, and use of cottonseed meal, hulls and linters in animal feed.

The genetic modification of cotton GHB614 is intended to improve agronomic performance only and is not intended to influence the nutritional properties, processing characteristics and overall use of cotton as a crop.

4.2.2. Effect of processing

Cotton GHB614 has been found to be compositionally equivalent to the non-GM comparator and other conventional cotton varieties except for the introduced trait (see Section 3.2.2).

The applicant provided data on the chemical compositions of cottonseed linters, hulls, delinted seeds, meal, toasted meal, crude oil and refined, deodorised oil obtained by

processing of cotton GHB614 and the non-GM counterpart. The amount of 2mEPSPS present in those materials is summarised in section 2.2.3.1. No nutritionally relevant differences were found (see Section 3.2.2). Taking into account the compositional analysis of whole linted cottonseed providing no indication of relevant compositional changes (see Section 3.2.2), the Panel has no reason to assume that the characteristics of cotton GHB614 and derived processed products would be different from those of the respective products derived from conventional cotton. Considering the toxicological profile and allergenic properties (see Sections 4.2.3, 4.2.4 and 4.2.5) the potential presence of the 2mEPSPS protein in processed products does not raise concern.

4.2.3. Toxicology

4.2.3.1. 2mEPSPS protein used for safety assessment

Due to the low expression level of the 2mEPSPS protein in cotton GHB614 and the very difficult task to isolate a sufficient quantity of purified protein from the genetically modified cotton, the safety studies with the newly expressed protein were conducted with a 2mEPSPS protein expressed in a recombinant *Escherichia coli* strain. The structural and functional equivalence of the 2mEPSPS protein produced by *E. coli* to that produced in cotton GHB614 was shown by N-terminal sequencing (Edman degradation), mobility in SDS-PAGE, Western analysis, HPLC/electrospray mass spectrometry (LC/MS) of peptides from a trypsin digest, glycosylation analysis and determination of 2mEPSPS enzymatic activity. Based on the identified similarity in structure and function between these proteins, the GMO Panel accepts the use of the 2mEPSPS protein derived from *E. coli* for the safety testing of the 2mEPSPS protein present in cotton GHB614.

4.2.3.2. Toxicological assessment of expressed novel protein

EPSPS enzymes occur in conventional plants, fungi and microorganisms and are thus consumed as part of the normal diet by humans and animals. No adverse effects associated with the intake of these proteins have been identified. The amino acid sequence of the 2mEPSPS protein is identical to that of the modified EPSPS (mEPSPS) protein expressed in GM maize event GA21, which has been previously evaluated by the EFSA GMO Panel and regarded as safe as its non-GM counterparts for human and/or animal consumption (EFSA, 2007a).

The 2mEPSPS protein expressed in cotton GHB614 (molecular mass ca. 47 kDa) is a modified version of the endogenous maize EPSPS protein. The amino acid sequence of the protein expressed in cotton GHB614 differs from that of the maize protein in 2 of the total of 445 amino acids. Threonine in position 102 of maize EPSPS has been replaced by isoleucine in 2mEPSPS, and proline in position 106 by serine, resulting in tolerance of the plants to glyphosate.

(a) Acute toxicity testing

In an acute oral toxicity study using mice, the 2mEPSPS protein produced in *E. coli* did not induce adverse effects after administration by gavage at a single dose of 2000 mg/kg bodyweight (bw). In addition, no systemic effects were induced when the protein was administered intravenously up to the highest dose of 10 mg/kg bw.

(b) Degradation in simulated digestive fluids

The digestibility of the 2mEPSPS protein was studied *in vitro* in simulated gastric fluid (SGF). No intact protein and no fragments were detectable after incubation for 30 seconds in pepsin-containing SGF at pH 1.2 as demonstrated by SDS-PAGE and protein staining.

Rapid degradation (within seconds) of the 2mEPSPS protein also occurred in simulated intestinal fluid (SIF) containing pancreatin at pH 7.5 as demonstrated by Western analysis.

(c) Bioinformatic studies

Bioinformatics-supported comparisons of the amino acid sequence of the 2mEPSPS protein expressed in cotton GHB614 with amino acid sequences contained in protein databases (dated 2004 and 2006) using the blastp algorithm indicated significant homology only with other EPSPS-related proteins. No sequence homology between the 2mEPSPS protein and known toxic proteins was found. On request of the EFSA GMO Panel the applicant provided an additional analysis using up-to-date protein databases (dated 2007 and 2008) and the FASTA sequence alignment tool, which confirmed the results of the previous study.

4.2.3.3. Toxicological assessment of new constituents other than proteins

No new constituent other than the 2mEPSPS protein is expressed in cotton GHB614 and no relevant changes in the composition of cotton GHB614 were detected in the comparative compositional analysis (see Section 3.2.2).

4.2.4. Toxicological assessment of the whole GM food/feed

On the basis of the comparative analysis the EFSA GMO Panel concluded that cotton GHB614 is compositionally and agronomically equivalent to the non-GM comparator and other conventional cotton varieties except for the introduced trait. In addition, this analysis as well as the molecular characterisation provided no indications of unintended effects of the genetic modification. According to the EFSA GMO Panel guidance document, animal safety studies with the whole food/feed are not required (EFSA, 2006a).

4.2.5. Allergenicity

Strategies used when assessing the potential allergenic risk focus on the characterisation of the source of the recombinant protein, the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons and whether the transformation may have altered the allergenic properties of the modified food. A weight-of-evidence approach is recommended, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (CAC, 2003; EFSA, 2006a).

4.2.5.1. Assessment of allergenicity of the newly expressed proteins

The *epsps* gene encoding the EPSPS protein was originally derived from maize, a source which is not regarded as a common allergenic food.

Bioinformatics-supported comparisons of the amino acid sequence of the 2mEPSPS protein with sequences of known allergens using databases dated 2004 and 2006 and the blastp algorithm were performed. A search for overall similarity indicated no similarity of 2mEPSPS with known allergenic proteins applying a criterion of 35% identity over a window of 80 amino acids. A search for identical sequences of at least 8 contiguous amino acids using the FindPatterns algorithm also showed no similarities between the 2mEPSPS protein expressed in cotton GHB614 and known allergens. Additional studies using up-to-date databases (dated 2007 and 2008) and the FASTA and, respectively, the FindPatterns algorithm, were provided on request of the EFSA GMO Panel and confirmed the previous results. Moreover, the protein is not glycosylated.

As described above, 2mEPSPS was rapidly degraded under simulated gastric and intestinal conditions (see Section 4.2.3.2.).

Based on this information, the EFSA GMO Panel considers that it is unlikely that the newly expressed 2mEPSPS protein in cotton GHB614 is an allergen.

4.2.5.2. Assessment of allergenicity of the whole GM plant

Allergenicity of the whole crop could be increased as an unintended effect of the random insertion of the transgene in the genome of the recipient, for example through qualitative or quantitative modifications of the pattern of expression of endogenous proteins. This issue does not appear relevant to the EFSA GMO Panel since cotton is not considered to be a common allergenic food. Furthermore, the main cottonseed product in human food, cottonseed oil, is highly purified and contains negligible levels of proteins, if any. Edible oils that are refined, bleached and deodorised do not appear to pose a risk to allergic individuals, as they contain virtually no proteins. The applicant states that no toxic or allergic effects on workers handling cotton GHB614 in the field since its first field release in 2002 have been reported.

Based on the available information, the EFSA GMO Panel concludes that it is unlikely that the overall allergenicity of the whole GM cotton GHB614 has been changed.

4.2.6. Nutritional assessment of GM food/feed

A 42-day feeding study using broiler chickens (Ross #708) was performed. Three groups of 140 animals consisting of 14 pens (7 pens/gender) with 10 animals each were fed diets containing toasted meal obtained from seeds of cotton GHB614 sprayed with glyphosate-based herbicides, the non-GM counterpart Coker 312 or another conventional non-GM variety, both treated with a different herbicide. The inclusion rate of cottonseed meal in the starter, grower and finisher diets was 10%. Although some statistically significant differences were noted among several determinations, mostly at specific time points, there were no relevant differences in body weight gain, feed consumption and feed conversion rate. There were also no relevant differences in weights of chilled carcass, abdominal fat pad, leg, thigh, wing and breast in animals fed meal derived from cotton GHB614 compared with animals fed meal from the non-GM conventional cotton varieties.

Thus, the broiler feeding study supported the results of the comparative compositional analysis which showed that seed from cotton GHB614 is compositionally and therefore nutritionally equivalent to the non-GM comparator and other conventional cotton varieties.

4.2.7. Post-market monitoring of GM food/feed

The risk assessment concluded that no data have emerged to indicate that cotton GHB614 is any less safe than its non-GM comparator. In addition, cotton GHB614 is, from a nutritional point of view, equivalent to conventional cotton. Therefore, and in line with the EFSA GMO Panel guidance document (EFSA, 2006a), the EFSA GMO Panel is of the opinion that post-market monitoring of the GM food/feed is not necessary.

4.3. Conclusion

The 2mEPSPS protein expressed in cotton GHB614 differs from the EPSPS protein present in conventional maize in 2 amino acids. The protein shows no homology to known toxic proteins and/or allergens. The 2mEPSPS protein was rapidly degraded in simulated gastric and intestinal fluid. This protein is also expressed in maize GA21 which has been previously assessed for its safety by the EFSA GMO Panel.

The comparative analysis showed no biologically relevant compositional, agronomic, and phenotypic changes of cotton GHB614 in relation to conventional cotton except for the introduced trait. A nutritional feeding study using broiler chickens indicated that seed from cotton GHB614 is nutritionally equivalent to seed from the non-GM counterpart and other conventional cotton. The study therefore supports the conclusion of the compositional and agronomical comparison that the genetic modification resulted in no unintended effects. The EFSA GMO Panel considers that no additional animal safety or nutritional wholesomeness study is needed. Based on the available information, the EFSA GMO Panel concludes that it is unlikely that the overall allergenicity of the whole cotton GHB614 has been changed. The EFSA GMO Panel is of the opinion that cotton GHB614 is as safe as its non-GM counterpart and other conventional cotton varieties.

5. Environmental risk assessment and monitoring

5.1. Issues raised by Member States

Issues raised by Member States are addressed in Annex G of the EFSA overall opinion.

5.2. Evaluation of relevant scientific data

5.2.1. Environmental risk assessment

The scope of application EFSA-GMO-NL-2008-51 includes import and processing for food/feed uses of cotton GHB614 and does not include cultivation. Considering the proposed uses of cotton GHB614, the environmental risk assessment is concerned with the indirect exposure through manure and faeces from gastrointestinal tracts mainly of animals fed on cotton GHB614 and with the accidental release into the environment of cotton GHB614 seeds during transportation and processing.

As the scope of the present application excludes cultivation, concerns regarding the use of glyphosate-based herbicides on cotton GHB614 apply only to imported and processed cotton products that may have been treated with these herbicides in countries of origin. The risk assessment of residues of this active ingredient falls within the scope of Directive 91/414/EEC concerning the placing of plant protection products on the market.

5.2.1.1. Potential unintended effects on plant fitness due to the genetic modification

Gossypium herbaceum and *G. hirsutum* are highly domesticated crops that have been grown in Southern Europe since the 19th century, giving rise to feral plants which can occasionally be found in the same area (Davis, 1967). The main cultivated cotton (*G. hirsutum*) is an annual self-pollinating crop. In the absence of insect pollinators (such as wild bees, honeybees, bumblebees), cotton flowers are self-pollinated, 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 seed dispersal are potential sources of vertical gene flow to cross-compatible wild cotton relatives, other cotton varieties and to occasional feral cotton plants. However, in Europe, there are no cross-compatible wild relatives with which cotton can hybridise. Because cotton pollen is very large (120 and 200 microns), heavy and sticky, wind-mediated dispersal of pollen to other cotton varieties is negligible (Vaissière 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; Van Deynze et al., 2005; Zhang et al., 2005; Hofs et al., 2007; Llewellyn et al., 2007). Seeds are thus the only survival structures.

The seed-mediated establishment of cotton and its survival outside of 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). Adequate soil moisture is an additional factor affecting the survival of feral cotton seedlings. Since general characteristics of cotton GHB614 are unchanged relative to its conventional counterpart, the inserted herbicide tolerance trait is not likely to provide a selective advantage outside of cultivation in Europe. If accidental release into the environment occurs, cotton GHB614 plants will only have a selective advantage in the presence of glyphosate-based herbicides which are not currently used on cultivated cotton or in most areas where the GM cotton might be spilled. It is thus considered very unlikely that cotton GHB614, or its progeny, will differ from other cotton varieties in their ability to survive until subsequent seasons or to establish feral populations under European environmental conditions. The risk of GM cotton becoming feral along transportation roads, or a weed on dairy farms where raw cotton seed is used as feed has been shown to be negligible in north-east Australia (Addison et al., 2007).

Data presented in the application gathered over a series of field trials across the US in 2004 and 2005 indicate that cotton GHB614 has no altered reproductive, dissemination or survivability characteristics compared to its conventional counterpart. 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 (Eastick and Hearnden, 2006; Bagavathiannan and Van Acker, 2008). There is no information to indicate change in survival capacity (including over-wintering). Furthermore,

there is no evidence that the herbicide tolerance trait introduced by the genetic modification results in increased persistence and invasiveness of any crop species, except in the presence of glyphosate-based herbicides. 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. In addition, the applicant states that cotton GHB614 will be imported as mostly non-viable seed. Therefore, the likelihood that some imported seed could escape and germinate is very low.

The EFSA GMO Panel is of the opinion that, even in case of accidental release into the environment, cotton GHB614 is very unlikely to show any enhanced fitness and would behave as conventional cotton.

5.2.1.2. Potential for gene transfer

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

(a) Plant to bacteria gene transfer

Based on current scientific knowledge and previous scientific opinions (EFSA, 2004) or statements (EFSA, 2007b), horizontal gene transfer from GM plants to microorganisms under natural conditions is extremely unlikely (Keese, 2008).

Transgenic DNA is a component of many food and feed products derived from GM cotton. Therefore, microorganisms in the digestive tract of humans and animals (domesticated animals and other animals feeding on fresh and decaying GM plant material) may be exposed to transgenic DNA.

In the case of accidental release and establishment of cotton GHB614 in the environment, exposure of microorganisms to transgenic DNA derived from GM cotton plants would take place during natural decay of GM plant material and/or pollen in the soil of areas where GM plants establish.

The modified *epsps* gene derives from wild type *epsps* maize gene. Taking into account the origin and nature of the *2mepsps* gene and the lack of selective pressure for this gene in the intestinal tract and/or the environment, the likelihood that horizontal gene transfer would result in increased fitness of microorganisms is very limited. For this reason, it is very unlikely that the *2mepsps* gene from cotton GHB614 would become transferred and established in the genome of microorganisms in the environment (including plant-associated microorganisms e.g., rhizobia) or human and animal digestive tract. In the very unlikely event that such a horizontal gene transfer would take place, no adverse effects on human and animal health and the environment are expected.

(b) Plant to plant gene transfer

Considering the intended uses of cotton GHB614 and physical characteristics of cotton seeds, a possible pathway of gene dispersal is from seed spillage and pollen of occasional feral GM cotton plants originating from accidental seed spillage during transportation and/or processing.

G. herbaceum is reported (Zohary and Hopf, 2000) to be a traditional fiber crop in the Eastern Mediterranean area already in the pre-Columbus period (before 1500 AD). The genus *Gossypium* consists of at least four species: *Gossypium arboreum*, *Gossypium barbadense*, *G. herbaceum* and *G. hirsutum*. 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 (Davis, 1967; 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 cotton GHB614 is restricted to cultivated and occasional feral populations. The EFSA GMO Panel also takes into account the fact that the present application does not include cultivation of cotton GHB614 within the EU so that the likelihood of cross-pollination between the imported cotton GHB614, other cotton crops and occasional feral cotton plants is considered to be extremely low. Even in case feral populations of cotton GHB614 were established or transgene flow occurred to cultivated and feral cotton, a selective advantage would only occur if the complementary glyphosate-based herbicides were applied.

5.2.1.3. Potential interactions of the GM plant with non-target organisms

Due to the intended uses of cotton GHB614, which exclude cultivation and due to the low level of exposure to the environment, potential interactions of the GM plant with non-target organisms were not considered an issue by the EFSA GMO Panel.

5.2.1.4. Potential interactions with the abiotic environment and biogeochemical cycles

Due to the intended uses of cotton GHB614, which exclude cultivation and due to the low level of exposure to the environment, potential interactions with the abiotic environment and biogeochemical cycles were not considered an issue by the EFSA GMO Panel.

5.2.2. Monitoring

Objectives of a monitoring 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 environmental risk assessment are correct, and (2) 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 related to risk management, and thus a final adoption of the monitoring plan falls outside the mandate of EFSA. However, the EFSA GMO Panel gives its opinion on the scientific quality of the monitoring plan provided by the applicant (EFSA, 2006a,b). The only significant exposure to the environment of the GM cotton would be through manure and faeces from the gastrointestinal tracts mainly of animals fed on the GM cotton or through accidental spillage of GM seeds during transportation and processing. The EFSA GMO Panel is aware that, due to physical characteristics of cotton seeds and methods of transportation, accidental spillage is unavoidable. Therefore, the EFSA GMO Panel recommends that appropriate management systems are introduced to actively monitor the occurrence of feral cotton plants in areas where seed spillage and plant establishment are likely to occur as proposed in the EFSA GMO Panel guidance document (EFSA, 2006a) and the scientific opinion of the EFSA GMO Panel on post-market environmental monitoring (EFSA, 2006b).

The scope of the monitoring plan provided by the applicant is in line with the intended uses for the GMO. Since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects, no case-specific monitoring is necessary.

The general surveillance plan proposed by the applicant includes (1) the description of an approach involving operators (federations involved in cotton import and processing), reporting to the applicants, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment, and (2) a coordinating system newly established by EuropaBio for the collection of the information recorded by the various operators. The applicant will submit a general surveillance report on an annual basis and a final report at the end of the consent. In case of confirmed adverse effects, the applicant will immediately inform the European Commission and Member States.

The EFSA GMO Panel is of the opinion that the scope of the monitoring plan provided by the applicant is in line with the intended uses of cotton GHB614 since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

5.3. Conclusion

Cotton GHB614 is being assessed for import and processing for food/feed uses and thus there is no requirement for scientific information on environmental effects associated with cultivation. Considering the intended uses, the environmental risk assessment is concerned with indirect exposure through manure and faeces from gastrointestinal tracts mainly of animals fed on the cotton GHB614 and with accidental spillage of GHB614 seeds during transportation and processing. The EFSA GMO Panel considered the environmental issues raised by Member States in the above sections of Chapter 5 and concludes as follows: *G. hirsutum*, which has no cross-compatible wild relatives in Europe, is a cultivated plant in Europe since the 19th century and occurs only occasionally as feral plants in Europe.

If accidental spillage and subsequent release into the environment of cotton GHB614 seeds occurs, cotton GHB614 plants will only have a selective advantage in the presence of glyphosate-based herbicides which are not currently used on cultivated cotton or in most areas where the GM cotton might be spilled. Therefore, the EFSA GMO Panel is of the opinion that the likelihood of the establishment and spread of cotton GHB614 is very low and that unintended environmental effects due to this GM cotton will be no different from that of other cotton varieties. Furthermore, the scope of the monitoring plan provided by the applicant is in line with the intended uses of cotton GHB614 since this does not include cultivation.

The EFSA GMO Panel is aware that, due to physical characteristics of cotton seeds and methods of transportation, accidental spillage is unavoidable. Therefore, the EFSA GMO Panel recommends that, within general surveillance, appropriate management systems are introduced to actively monitor the occurrence of feral cotton plants in areas where seed spillage is likely to occur.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The EFSA GMO Panel was requested to carry out a scientific risk assessment of the cotton GHB614 for food and feed uses, import and processing.

Cotton GHB614 has been modified to express a modified *epsps* maize gene providing tolerance to glyphosate-based herbicides. The EFSA GMO Panel is of the opinion that the molecular characterisation provided for cotton GHB614 is sufficient for the safety assessment. The bioinformatic analysis of the inserted DNA and flanking regions does not raise any safety concern. The expression of the gene introduced by genetic modification has been sufficiently analysed and the stability of the genetic modification has been demonstrated over several generations. The EFSA GMO Panel considers that the molecular characterisation does not indicate any safety concern.

Comparative analysis has shown that cotton GHB614 is compositionally and agronomically equivalent to conventional cotton, except for the introduced trait. The risk assessment included an analysis of data from analytical studies, bioinformatics, and *in vitro* and *in vivo* studies. Based on the available information, the EFSA GMO Panel concludes that it is unlikely that the overall allergenicity of the whole cotton GHB614 has been changed. The EFSA GMO Panel is of the opinion that cotton GHB614 is as safe as its non-GM counterpart and other conventional cotton varieties.

The application EFSA-GMO-NL-2008-51 concerns import, processing and food/feed uses. There is therefore no requirement for scientific information on possible environmental effects associated with the cultivation of cotton GHB614. Considering the scope of the application, not for cultivation, the EFSA GMO Panel is of the opinion that the likelihood of the spread and establishment of cotton GHB614 is very low and that unintended environmental effects due to this cotton will be no different from that of other cotton varieties. The scope of the monitoring plan provided by the applicant and the reporting intervals are in line with the intended uses of cotton GHB614. However, the EFSA GMO Panel is aware that, due to the physical characteristics of cotton seeds and methods of transportation, accidental spillage is unavoidable. Therefore, the EFSA GMO Panel recommends that, within general surveillance, specific measures are introduced to actively monitor the occurrence of feral cotton plants in areas where seed spillage is likely to occur.

In conclusion, the EFSA GMO Panel considers that information available for cotton GHB614 addresses the outstanding questions raised by the Member States and considers it unlikely that cotton GHB614 will have any adverse effect on human and animal health or on the environment in the context of its proposed uses.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of the Netherlands, dated 25 January 2008, concerning a request for placing on the market of cotton GHB614 in accordance with Regulation (EC) No 1829/2003.

2. Acknowledgement letter, dated 30 January 2008, from EFSA to the Competent Authority of the Netherlands (Ref SR/KL/shv (2008) 2649496).

3. Letter from EFSA to applicant, dated 11 March 2008, delivering the 'Statement of Validity' for application EFSA-GMO-NL-2008-51, cotton GHB614 submitted by Bayer CropScience under Regulation (EC) No 1829/2003 (Ref SR/AC/shv (2008) 2757434).

4. Letter from EFSA to applicant, dated 3 September 2008, requesting additional information and stopping the clock (Ref PB/YD/md (2008) 3272819).

5. Letter from applicant to EFSA, dated 1 April 2008, providing the timeline for submission of response.

6. Letter from EFSA to applicant, dated 12 November 2008, requesting additional information and maintaining the clock stopped (Ref PB/YD/shv (2008) 3449720).

7. Letter from applicant to EFSA, dated 1 December 2008, providing additional information.

8. Letter from applicant to EFSA, dated 7 January 2009, providing the timeline for submission of response.

9. Letter from applicant to EFSA, dated 12 January 2009, providing additional information.

10. Letter from EFSA to applicant, dated 24 February 2009, restarting the clock (Ref PB/YD/shv (2009) 3698443).

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