Opinion of the Scientific Panel on Genetically Modified Organisms on an application (Reference EFSA-GMO-NL-2005-13) for the placing on the market of glufosinate-tolerant genetically modified LLCotton25, for food and feed uses, and import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience[1]

(Question No EFSA-Q-2005-047)

Opinion adopted on 6 December 2006

SUMMARY

This document provides an opinion of the Scientific Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority (EFSA) on genetically modified LLCotton25 (Unique Identifier ACS-GHØØ1-3) developed to provide tolerance to glufosinate-containing herbicides.

In delivering its opinion the GMO Panel considered the application EFSA-GMO-NL-2005-13, additional information provided by the applicant (Bayer CropScience) and the scientific comments submitted by the Member States. The application EFSA-GMO-NL-2005-13 covers the import and processing of LLCotton25 seeds and its derived products for use as food (e.g. oil, linters) and/or feed (e.g. meal, hulls, oil). The GMO Panel assessed LLCotton25 with reference to the intended uses and the 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 characterization of the inserted DNA and expression of the target protein. A comparative analysis of agronomic traits and composition was undertaken and the safety of the new protein and the whole food/feed was evaluated with respect to potential toxicity and allergenicity. Both a nutritional and an environmental assessment, including a monitoring plan, were undertaken.

LLCotton25 is derived from the cotton variety Coker312 which was transformed by *Agrobacterium*-mediated gene transfer technology. LLCotton25 expresses the *bar* gene leading to the production of the enzyme, phosphinothricin acetyl-transferase (PAT) that acetylates L-glufosinate-ammonium. The PAT enzyme confers tolerance to glufosinate-containing herbicides (trade names: Liberty®, Basta®).

Molecular analysis shows that LLCotton25 contains a single insert and does not retain backbone sequences from the vector. The GMO Panel is of the opinion that bioinformatic analysis of the DNA insert and flanking regions indicates no cause for concern, and that sufficient evidence for the stability of the insert structure was provided.

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Compositional and agronomic analyses indicate that the LLCotton25 was compositionally and agronomically equivalent to other tested conventional cotton lines, except for the introduced transgenic trait. The comparative analysis of LLCotton25 therefore provides no indication for unintended effects resulting from the genetic modification. The GMO Panel is therefore of the opinion that the LLCotton25 is as safe as its non genetically modified counterparts.

The application EFSA-GMO-NL-2005-13 concerns import, processing and food/feed uses. There is therefore no requirement for scientific information on possible environmental effects associated with the cultivation of LLCotton25. The GMO Panel agrees that unintended environmental effects due to the establishment and spread of LLCotton25 will not be different from that of conventionally bred cotton.

Considering the intended uses of LLCotton25, the monitoring plan provided by the applicant is in line with the EFSA Guidance document and the opinion of the GMO Panel on post-market environmental monitoring. However the GMO Panel is aware that, due to the physical characteristics of cotton seeds and methods of transportation, accidental spillage is unavoidable. Therefore the 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 GMO Panel considers that the information available for LLCotton25 addresses the scientific comments raised by the Member States and that the GM LLCotton25 is as safe as its non genetically modified counterparts with respect to potential effects on human and animal health or the environment. Therefore the GMO Panel concludes that LLCotton25 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: GMO, cotton, LLCotton25, glufosinate tolerance, food/feed safety, PAT protein, *bar* gene, PAT protein, ACS-GHØØ1-3, human and animal health, environment, import, Regulation (EC) No 1829/2003.

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BACKGROUND

On 7 March 2005 EFSA received from the Dutch Competent Authority an application (Reference EFSA-GMO-NL-2005-13), for authorisation of LLCotton25 (Unique Identifier ACS-GHØØ1-3), submitted by Bayer CropScience within the framework of Regulation (EC) No 1829/2003 on genetically modified food and feed (EC, 2003).

After receiving the application EFSA-GMO-NL-2005-13 and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed the 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 5 August 2005 EFSA received additional information (requested on 14 July 2005) and declared the application as formally valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003 on 2 September 2005.

EFSA made the valid application available to Member States and the European Commission and consulted nominated risk assessment bodies of the Member States, including the national Competent Authorities within the meaning of Directive 2001/18/EC (EC, 2001) following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. The Member State bodies had three months after the date of receipt of the valid application (until 2 December 2005) within which to make their opinion known.

On 26 January 2006 the GMO Panel asked for additional data on the compositional analysis of LLCotton25. The applicant provided the complete requested information on 18 May 2006. After receipt and assessment of the full data package, the GMO Panel finalized its risk assessment of LLCotton25.

The GMO Panel carried out a scientific assessment of the genetically modified (GM) cotton LLCotton25 for food and feed uses and 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 the Member States and the additional information provided by the applicant.

In giving its opinion on LLCotton25 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 receipt of the valid application. As additional information was requested by the EFSA GMO Panel, the time-limit of 6 months was extended accordingly, in line with Articles 6(1), 6(2), 18(1), and 18(2) of Regulation (EC) No 1829/2003.

According to Regulation (EC) No 1829/2003, 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 overall opinion in accordance with Articles 6(5) and 18(5).

TERMS OF REFERENCE

The GMO Panel was requested, in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003, to carry out a scientific assessment of the genetically modified LLCotton25 for import, processing and food/feed uses.

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 GMO Panel was not requested to give an opinion on information required under Annex II to the Cartagena Protocol. The 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.

ASSESSMENT

1. Introduction

The genetically modified (GM) LLCotton25 (Unique Identifier ACS-GHØØ1-3) was assessed with reference to its intended uses, taking account of the 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 (EFSA, 2006a).

2. Molecular characterisation

2.1. Issues raised by the Member States

Questions were raised regarding (1) the putative deletions of plant DNA sequences which occurred as a consequence of the insertion and (2) the need for further data regarding such deletions (e.g. further transcriptional analysis).

Comments raised by the Member States on specific molecular detection methodologies as well as on their validation are not in the remit of the GMO Panel.

2.2. Evaluation of relevant scientific data

2.2.1. Transformation process and vector constructs

Explants of *Gossypium hirsutum* from variety Coker312 were transformed by the vector plasmid pGSV71 using *Agrobacterium tumefaciens* disarmed strain C58C1^{Rif}. The vector pGSV71 is derived from pGSC1700 and contains the origin of replication (ColE1) from pBR322 for replication in *E. coli*, the origin of replication from the *Pseudomonas* plasmid pVS1 for replication in *Agrobacterium tumefaciens*, the *aadA* gene conferring resistance to streptomycin

and spectinomycin, and a T-DNA region containing a multiple cloning site and the right and left border sequences from pTib6S3.

An EcoRI/HindIII fragment inserted into the multiple cloning site comprises the following elements: the P35S3 region containing the Cauliflower Mosaic Virus 35S promoter, the *bar* gene from *Streptomyces hygroscopicus* ATCC21705 coding for glufosinate-ammonium tolerance, and the 3'nos terminator sequence including the 3' untranslated region of the nopaline synthase gene from the T-DNA of pTiT37. Although the *bar* gene, commonly present in the nature/microorganisms, starts with a GTG initiation codon, the N-terminus of the *bar* coding region in LLCotton25 was modified to obtain an ATG initiation codon, thereby ensuring correct translation initiation in plants. Additionally, the second codon of the *bar* gene (AGC encoding serine) has been modified to GAC (encoding aspartic acid) prior to transformation.

The expression of the *bar* gene leads to the production of the enzyme, phosphinothricin acetyl-transferase (PAT) that acetylates L-glufosinate-ammonium and thereby confers tolerance to glufosinate-containing herbicides (trade names, Liberty®, Basta®).

2.2.2. Transgenic constructs in the genetically modified plant

Southern analysis of genomic DNA digested with five different restrictions enzymes using the entire T-DNA as a probe showed the presence of a single insertion locus. The absence of vector backbone sequences in LLCotton25 plants has been confirmed by Southern analysis using four overlapping probes that cover the entire vector backbone. Thereby it was confirmed that the *aadA* gene has not been transferred to LLCotton25.

The nucleotide sequence of the insert introduced into LLCotton25 has been determined in its entirety. The DNA sequence of the LLCotton25 insert has been proven to be identical to the corresponding transforming plasmid pGSV71 sequences. PCR analysis of the terminal repeats of the vector plasmid confirmed that the right border (RB) terminal repeat is not completely integrated in LLCotton25 as 23 bp are missing. The left border (LB) terminal repeat sequence displays a deletion of 4 bp. The sequences of the plant genome adjacent to the 3' and 5' sequences of the insert were determined using TAIL-PCR. Comparison of the flanking sequences to the respective wild type target site revealed that upon integration of the T-DNA into the genomic DNA a 38 bp fragment of genomic DNA at the target site was deleted. There was no indication that the insert is integrated in a coding region or that the insert disrupts gene regulatory sequences. These data presented proof that the insert has been integrated in a single chromosome at a single locus as intended.

2.2.3. Information on the expression of the insert

2.2.3.1. Expression of the introduced genes

Transcription of the bar gene was analysed by Northern analysis and detected in leaves, stems, roots and seeds. Pollen was not analysed. Analysis of PAT protein expression was carried out by ELISA using plants grown under greenhouse conditions. The tissues and plant samples examined were stems, roots, seeds, leaves and pollen from glufosinate-treated and untreated LLCotton25 plants. The PAT protein could be detected in all transgenic tissues mentioned. The level of PAT protein accumulation was measured as PAT protein content of total extractable protein in the following order for different tissues: leaves and stems more than roots much more than seeds and pollen.

[The average amount of PAT protein in four growth stages of the life cycle of the plant (2-4 leaf-stage, the 4-6 leaf-stage, beginning of bloom and full bloom stages) ranged from about 58 to 98 µg PAT/g fresh weight in both, the glufosinate-treated and untreated GM leaf samples. The PAT protein content declines in the latter growth stage in leaves of both treated and untreated LLCotton25. PAT protein comprised an average of 0.21-0.35% of the total crude protein in the leaves of LLCotton25.

Furthermore, field trials at different locations showed that the expression levels of the PAT protein in cotton seeds was of the same order of magnitude as found in leaves.

2.2.3.2. Putative cryptic open reading frames (ORF) in LLCotton25

Bioinformatic analysis (BLAST searches) of the LLCotton25 insert sequence indicates the presence of 26 putative open reading frames (ORFs for putative peptides of a size of 4 to 93 aa) spanning the junctions between the DNA of the nuclear cotton genome and the inserted DNA. This raises the possibility that new putative fusion proteins could be produced. Further analysis revealed that three ORF's were found at the 3' junction region of the insert that potentially could give rise to putative peptides. Bioinformatic analysis of these three ORF-sequences showed no sequence homology with known toxins or allergens. These results do not raise any safety concerns.

2.2.4. Inheritance and stability of inserted DNA

LLCotton25 was developed from cotton line Coker312 by *Agrobacterium*-mediated gene transfer technology. The inheritance of the introduced trait follows a Mendelian pattern. The LLCotton25 event has also been introduced into different genetic backgrounds (FiberMax966, FiberMax832 and FiberMax989, picker varieties; HS26 and AVS9023, stripper varieties). Such seeds with the LLCotton25 event in different genetic backgrounds were grown under greenhouse conditions and treated by a standard spray test using the herbicidal agent glufosinate-ammonium. The results confirmed phenotypically the presence of the herbicide-tolerant trait and indicated the presence of the functional PAT protein. DNA from individual plants of both LLCotton25 in different genetic backgrounds and its non GM counterparts was subjected to Southern analysis with a probe specific for the insert. Interpretation of the banding patterns from various restriction enzyme digests of the DNA of LLCotton25 in different genetic backgrounds demonstrated the stability at the genetic level over multiple generations.

The same kind of analysis was performed with genomic DNA isolated from plants of generation T6 grown at 11 different locations (i.e. different environmental conditions). The DNA was isolated and digested by a restriction enzyme (NcoI) recognizing two restriction sites within the inserted DNA. The entire T-DNA employed as probe for Southern analysis detected the expected banding pattern in all samples analysed including the two bands representing the junctions between the inserted DNA and the genomic plant DNA. These findings demonstrate the molecular stability of the transformation event LLCotton25 under different environmental conditions.

These results indicated phenotypic, genetic and molecular stability of the insert present in the LLCotton25 event in different genetic backgrounds, over several generations and under different environmental conditions.

2.3. Conclusion

The molecular characterisation data establish that LLCotton25 contains a single insert. The insert in LLCotton25 is constituted by the predicted and verified genetic elements present in the T-DNA in the transformation vector and does not contain genes from the vector backbone sequences. In addition analysis of ORFs spanning the two junction regions in the genetically modified cotton was performed by bioinformatic analysis. Bioinformatic analysis showed that, in the event that the three putative ORFs in the 3' region are expressed, any resulting polypeptides would show no significant sequence homology with known toxins or allergens.

The GMO Panel is of the opinion that the molecular characterisation of the DNA insert and flanking regions of LLCotton25 does not raise any safety concerns, and that sufficient evidence for the stability of the insert structure was provided.

3. Comparative analysis

3.1. Issues raised by Member States

Questions were raised regarding (1) the validity of the statistical analysis of the compositional data, (2) including the need for a statistical analysis of compositional data separately for each growing season and location.

3.2. Evaluation of relevant scientific data

Having considered the information provided in the application and the Member States comments, the GMO Panel requested from the applicant further data with respect to the statistical analysis of the compositional data as well as on the range of the gossypol content. The applicant provided an additional statistical analysis that the GMO Panel found adequate.

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

For compositional studies, LLCotton25 was compared to its parent variety, Coker312 which is a commercial cotton variety grown in the Southern US since 1990. The comparison also included data from the scientific literature regarding the natural ranges of key compounds in conventional cotton. Field trials were performed in year 2000 and 2001 in Arkansas, Georgia, Mississippi, Missouri, North-Carolina and Texas, all belonging to the cotton growing regions of Southern United States. Each year trials were performed at 15 locations, three treatments at each location and three replications per treatment (except in one site where the sample plot was harvested three times per treatment). One site was excluded for the analysis of fatty acids as different methods had been used for the different treatment samples within the site. The three 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 glufosinate-ammonium (Liberty®) herbicide weed control. Isolation distances of 12 m were maintained in order to avoid cross-pollination and herbicide treatment drift.

3.2.2. Compositional analysis

Materials were collected from the field trials for a compositional analysis of seeds and lint. 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, crude fibre, 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). Lint samples were only analysed for proximates.

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. In addition to comparing the composition of LLCotton25 with that of the non-GM parent variety, Coker312, the composition of the GM cotton was also compared to data from commercial cotton lines available in the literature (see Section 3.2.1.). The GMO Panel found the presentation of data adequate.

Although the PAT protein was detected at low amounts varying from 0.13 to 0.44 µg/g fresh weight (FW) in some non-GM seed samples (1 sample out of 4 in year 2000 and 5 out of 27 kernel samples in year 2001 with levels ranging from 0,132 µg/g to 0,365 µg/g FW), data from all control samples were used in the statistical evaluation of the composition of LLCotton25 as compared to Coker312. For comparison, the level of PAT protein in LLCotton25 seeds is 61.3–74.1 µg/g FW. The low level of the PAT protein in the control material is unlikely to have an impact on the outcome of the comparative compositional analysis and the GMO Panel therefore accepts the use of this control material.

The compositional comparisons occasionally revealed statistically significant differences of some compounds. In the analysis per site statistically significant differences were observed for a number of fatty acids i.e. myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid and linoleic acid analysed in seeds. However, the reported levels all fell well within the natural ranges reported in the literature (OECD, 2004). In the analysis per year statistically significant differences were also observed in compounds i.e. calcium, total gossypol and aspartic acid analysed in seeds. For most of these compounds the differences were small and within the natural ranges reported in literature. Only the free gossypol levels (a toxicant) in both the GM LLCotton25 and the non-GM comparator fell outside the natural ranges reported in the literature. The applicant was approached for an explanation. In reply, the applicant presented an additional statistical analysis that showed that there were no significant differences in free gossypol levels between LLCotton25 and the non-GM comparator when analysed over the 15 sites tested, and that the levels fell within ranges reported in the ILSI (International Life Science Institute) crop composition database (http://www.cropcomposition.org/).

The GMO Panel considered the observed compositional differences between LLCotton25 and its comparator in the light of the field trial design, measured biological variation and the level of the studied compounds in conventional cotton varieties, and concluded that LLCotton25 can be considered to have a composition equivalent to the non-GM counterpart and other conventional cotton lines, 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 USA, Australia and Brazil during multiple seasons. The characteristics that were analyzed in these studies included parameters related to plant morphology, seeds and plant development, reproductive traits, disease and pest susceptibility, weediness, weed control, volunteers, yields, cotton seed and fibre quality.

The GMO Panel noted that differences were observed in some instances with regard to several characteristics related to plant density, fibre quality, and phenotype (plant, seed, and flower). However these differences did not occur consistently in the various studies and, therefore, were not considered to be related to the genetic modification. The GMO Panel concludes that LLCotton25 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 glufosinate-treated and conventionally treated LLCotton25, its non-GM counterpart Coker312 and other conventional cotton lines indicated that the LLCotton25 was compositionally and also agronomically equivalent to conventional cotton lines, except for the introduced transgenic trait. The comparative analysis of LLCotton25 therefore provided no indication for unintended effects resulting from the genetic modification.

4. Food/Feed safety assessment

4.1. Issues raised by Member States

Questions were raised regarding the need for further animal feeding studies, such as a 90-day subchronic toxicity study in rats, nutritional studies in ruminants, as well as allergenicity studies.

4.2. Evaluation of relevant scientific data

4.2.1. Product description and intended use

The scope of application EFSA-GMO-NL-2005-13 includes the import and processing of LLCotton25 and its derived products for use as food/feed. Thus the possible uses of LLCotton25 includes the production of refined oil from seeds and cellulose from linters for use as human food, and use of cottonseed meal (or cake), hulls and linters in animal feed.

4.2.2. Stability during processing

Since LLCotton25 has been found to be compositionally equivalent to conventional cotton, except for the newly expressed trait (see Section 3.2.2), the stability during processing is not expected to be different from conventional cotton varieties.

4.2.3. Toxicology

4.2.3.1. PAT protein used for safety assessment

Due to the low expression level of the PAT protein in LLCotton25 most of the safety studies were conducted with a PAT protein encoded in *E. coli* by the *bar* gene (PAT/*bar* protein). Examination of the structure and function of these plant and bacterial PAT proteins have shown a high degree of similarity, based on their size and sequence homology, enzymatic activity, immunoreactivity and absence of glycosylation. The PAT/*pat* and PAT/*bar* proteins have been shown to be structurally and functionally equivalent (Wehrmann *et al.*, 1996; Herouet *et al.*, 2005). Therefore the GMO Panel accepts the PAT/*bar* as well as the PAT/*pat* test material derived from *E. coli* for the safety assessment of PAT protein present in LLCotton25.

4.2.3.2. Toxicological assessment of expressed novel protein in LLCotton25

(a) Acute and repeated short term toxicity testing

The applicant provided data on an acute toxicity study in mice with a PAT protein encoded by the *bar* gene generated in *E. coli*. Because of the expected fast proteolytic degradation in digestive environments, the potential toxicity of the protein was studied after intravenous injection at the dose levels of 1 and 10 mg/kg body weight. Even at the relatively high dose of 10 mg/kg body weight, no signs of systemic toxicity were observed.

No oral toxicity studies with the *bar* encoded PAT protein are available in this application. However, a 14-day repeated dose feeding study conducted in rats with the PAT protein encoded by the *pat* gene was provided. Groups of five male and female Wistar rats (HanIbm:WIST) received diets containing the PAT protein (lyophilized powder) at levels of 0 (group 4), 5 (group 2) and 50 (group 3) g/kg diet. The high level corresponded to a dose of 7.6 and 7.9 mg/kg BW/day for males and females, respectively. A second control group (group 1) was fed a standard rodent diet. In the study there were no remarkable findings apart from statistically significant increases in blood cholesterol levels (males of groups 2 and 3) and phospholipid levels (females of group 3 and males of groups 2 and 3). These effects, which also occurred in one of the control groups (group 4), are not regarded as toxicologically relevant. In conclusion, feeding the PAT protein to rats for 14 days revealed no indications for adverse effects up to the highest dose tested.

(b) Degradation in simulated digestive fluids

The PAT/bar protein expressed in *E. coli* was used in the degradation studies. The PAT protein was tested for *in vitro* digestibility in simulated gastric fluid containing pepsin. Degradation occurred rapidly, as shown by polyacrylamide gel electrophoresis (within 30 seconds at pH 2). Rapid degradation was also demonstrated by western blots in simulated intestinal fluid (pH 7.5) in presence of pancreatin. During degradation fragments of 7 kD appeared transiently. These fragments disappeared after 5 minutes of incubation. These *in vitro* digestion experiments demonstrate that the PAT protein encoded by the *bar* gene is rapidly degraded in simulated gastric and intestinal conditions.

(c) Bioinformatic studies

Searches for sequence homology between the *bar* gene encoded PAT protein in LLCotton25 and other proteins indicated significant homology only with other acetyltransferases. No sequence homology with known toxins was shown.

4.2.3.3. Toxicological assessment of new constituents other than proteins

No new constituent other than the PAT protein is expressed in LLCotton25 and no relevant changes in composition were detected by the compositional analysis.

4.2.4. Toxicological assessment of the whole GM food/feed

The comparative compositional analysis and agronomic analyses showed that LLCotton25 is substantially equivalent to its non-GM counterpart Coker312 and other commercially grown cotton varieties except for the introduced trait. In addition, the analyses provided no indication for unintended effects of the genetic modification and therefore the GMO Panel concluded that no additional safety studies with laboratory animals are needed.

4.2.5. Allergenicity

The 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).

4.2.5.1. Assessment of allergenicity of the newly expressed proteins

Potential expression products were analysed for possible homology to known allergens. The total amino acid sequence of the PAT/*bar* protein was compared to that of known allergens. The results of a linear epitope homology search over 8 contiguous amino acids showed no similarities between epitopes of known allergens and the PAT protein expressed by LLCotton25. Further, bioinformatic search with 80 amino acids window indicated no similarity with potential allergenic proteins applying a 35 % identity criterion. Based on these results PAT protein presented a high structural similarity only with non-allergenic PAT proteins, while no evidence for any homology to known toxic or allergenic proteins was found. Searches for potential N-glycosylation sites, which are often found on allergens, were negative. PAT is not stable in an acidic environment and is rapidly degraded under simulated gastric and intestinal conditions. It is also rapidly degraded and inactivated in stomach fluids of cattle and pig. Based on these results the GMO Panel considers that the newly expressed PAT protein is not likely to be allergenic.

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 GMO Panel since cotton (*Gossypium hirsutum* L.) is not considered to be an allergenic food. Furthermore, the main cotton seed product in human food, cotton seed 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 in workers handling LLCotton25 in the field since its first field release in 1999 have been reported.

The GMO Panel concludes that the information presented confirms that the overall allergenicity of the whole plant is not changed.

4.2.6. Nutritional assessment of GM food/feed

A total of 560 Ross commercial one-day old broiler chicks were used in a 33-day study to evaluate the nutritional characteristics of cotton seed meal derived from LLCotton25. The study consisted of four treatments in which 10% of the diet consisted of cotton seed meal derived from either LLCotton25 not treated with glufosinate-containing herbicide, LLCotton25 treated with glufosinate-containing herbicide, the near isogenic counterpart (Coker312), or a commercial variety. There were 10 birds and 14 replicates in each treatment group. There were no statistically significant differences between treatments in total feed consumption, total live-weight gain, and feed conversion efficiency. Although the thigh and breast weight from broilers fed the diet containing cotton seed meal from LLCotton25 not treated with glufosinate-containing herbicide was significantly lower when compared with the values for broilers receiving cotton seed meal from the commercial variety, there were no statistically significant differences in any of the weight variables between chickens fed the diet containing cotton seed meal from LLCotton25 treated with herbicide and the other three dietary treatments. These results indicate that the cotton seed meal derived from LLCotton25 treated with glufosinate-containing herbicide is nutritionally comparable with its near isogenic non-GM counterpart and the commercial varieties included in the study.

As the extensive comparative compositional analysis of LLCotton25 provided no indication for unintended effects of the genetic modification under consideration in this opinion, the GMO Panel concluded that no additional safety or nutrition study with laboratory animals is needed.

4.2.7. Post-market monitoring of GM food/feed

The risk assessment concluded that no data have emerged to indicate that LLCotton25 is any less safe than its non-GM comparator. In addition, LLCotton25 is, from a nutritional point of view, substantially equivalent to conventional cotton. Therefore, and in line with the Guidance document (EFSA, 2006a), the GMO Panel is of the opinion that post-market monitoring of the GM food/feed is not necessary.

4.3. Conclusion

No toxicity of the PAT protein was observed in the 14-day repeated dose feeding study conducted in rats and in the acute toxicity study in mice after intravenous injection. The PAT protein is rapidly degraded in simulated gastric and intestinal conditions. The PAT protein shows no homology with known toxins and/or allergens. An extensive compositional analysis showed no consistent compositional differences to conventional cotton with relation to key nutrients and anti-nutrients. A 33-day feeding study with broiler chickens did not provide any indications that the cotton seed meal derived from LLCotton25 treated with glufosinate-containing herbicides is nutritionally different from meal produced from its near isogenic non-GM counterpart or commercial varieties included in the study. The GMO Panel considers that no additional animal safety or nutritional study is needed. The GMO Panel is therefore of the opinion that the LLCotton25 is as safe as its non GM counterparts and that the overall allergenicity of the whole plant is not changed.

5. Environmental risk assessment and monitoring plan

5.1 Issues raised by Member States

Questions were raised regarding (1) the interactions of LLCotton25 with the biotic environment, (2) the need for more information on the application of herbicides, from season to season and in all the intended LLCotton25 growing countries, (3) the cotton-weeds for those member countries of the EC, where cotton is cultivated, (4) the gene-environment interactions, unintended or pleiotropic effects and (5) the need for data on the overwintering capacity of LLCotton25 and its parental variety Cocker 312 seeds.

Further comments were raised with respect to the environmental monitoring plan regarding (6) the need for an updated case-specific monitoring plan and (7) a more detailed general surveillance plan.

5.2. Evaluation of relevant scientific data

5.2.1. Environmental risk assessment

The scope of application EFSA-GMO-NL-2005-13 includes import, processing and food/feed uses of LLCotton25. Considering the proposed uses of LLCotton25, excluding cultivation purposes, the environmental risk assessment is limited to unintentional release into the environment of GM seeds during transportation and processing or when cotton seeds are used as food or feed.

As this application is not for cultivation, concerns regarding the use of glufosinate-containing herbicides on LLCotton25 apply only to imported and processed cotton products that may have been treated with those herbicides in the countries of origin. However the GMO Panel is aware that glufosinate-containing herbicides are used in Europe on other crops and that the risk assessment of such compounds is 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 *Gossypium hirsutum* are highly domesticated crops which 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; Todaro, 1917). There are no wild relatives in Europe. The main cultivated cotton (*Gossypium hirsutum* L.) is an annual self-pollinating crop which has a relatively low percentage of cross-pollination (Xanthopoulos & Kechagia, 2000; Turley & Kloth, 2002). Seed and pollen dispersal are potential sources of gene flow to conventional varieties and to occasional feral cotton plants. Cotton pollen is heavy and sticky so that the natural crossing is made mostly by insect pollinators (wild bees, honeybees, etc). Seeds are the only survival structures.

However, if accidental release into the environment occurs, these GM cotton plants will only be fitter in the presence of glufosinate-containing herbicides which are not currently used on cultivated cotton or in most areas where the GM cotton might be spilled.

In addition to the data presented by the applicant, the GMO Panel is not aware of any scientific report of increased fecundity or ferality of herbicide tolerant cotton in regions where GM cotton is cultivated. There is no information to indicate change in survival capacity (including

overwintering). Furthermore there is no evidence that the herbicide tolerance trait introduced by genetic modification result in increased invasiveness of any crop species, except in the presence of the herbicide. 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. The GMO Panel is thus of the opinion that, even in case of accidental release into the environment, LLCotton25 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 transfer/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 present scientific knowledge and elaborated in more detail elsewhere (EFSA, 2004), gene transfer from GM plants to microorganisms under natural conditions is extremely unlikely, and its establishment would occur primarily through homologous recombination in microorganisms.

Transgenic DNA is a component of some or most of the food and feed products derived from the 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.

The *bar* gene is known to be ubiquitous in soil microbial populations. Taking into account the origin and nature of *bar* gene and the lack of selective pressure in the intestinal tract, the likelihood that horizontal gene transfer would confer selective advantages or increased fitness of microorganisms is very limited. For this reason it is very unlikely that *bar* gene from LLCotton25 would become established in the genome of microorganisms in the environment 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 as no new traits would be introduced into microbial communities.

(b) Plant to plant gene transfer

Considering the intended uses of LLCotton25 and the physical characteristics of cotton seeds, a possible pathway of dispersal is from seed spillage and pollen of occasional feral GM cotton plants originating from accidental seed spillage during transportation and/or processing.

Gossypium herbaceum is reported (Zohary and Höpf, 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 crop species: *G. arboreum*, *G. 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; Todaro, 1917; Tutin *et al*., 1992; Zangheri, 1976) 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 populations. The 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

LLCotton25 were established or transgene flow occurred to cultivated and feral cotton, a selective advantage would only occur if the complementary glufosinate-containing herbicides were applied.

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

Because the level of exposure to PAT protein is so low, potential effects on non-target organisms are considered by the GMO Panel as very unlikely.

5.2.1.4. Potential interaction with the abiotic environment and biogeochemical cycles

Because the level of exposure to PAT protein is so low, potential effects on the abiotic environment and biogeochemical cycles are considered by the GMO Panel as very unlikely.

5.2.2. 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. 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.

General surveillance is related to risk management, and thus a final adoption of the general surveillance plan falls outside the mandate of EFSA. However, the GMO Panel gives its opinion on the scientific quality of the general surveillance plan provided by the applicant (EFSA, 2006a). The only significant exposure of the environment to the genetically modified cotton would be related to accidental spillage. The GMO Panel is aware that, due to the physical characteristics of cotton seeds and methods of transportation, accidental spillage is unavoidable. Therefore the 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 as proposed in the EFSA Guidance document (EFSA, 2006a) and the opinion of the GMO Panel on post-market environmental monitoring (EFSA, 2006b).

In other respects the GMO Panel is of the opinion that the general approaches and measures of the monitoring plan proposed by the applicant are in line with the EFSA opinion on post-market environmental monitoring (EFSA, 2006b) as well as with the intended uses of LLCotton25. Since the environmental risk assessment does not cover cultivation and identifies no potential adverse environmental effects, no case-specific monitoring is necessary.

The GMO Panel agrees with the proposal made by the applicant on the reporting intervals.

5.3. Conclusion

LLCotton25 is being assessed for import, processing and food/feed uses and thus there is no requirement for scientific information on environmental effects associated with cultivation. The GMO Panel considered the environmental issues raised by Member States in the above sections of Chapter 5 and concludes as follows: *Gossypium hirsutum* L., which has no 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 LLCotton25 seeds occurs, LLCotton25 plants will only be fitter in the presence of glufosinate-containing herbicides which are not currently used on cultivated cotton or in most areas where the GM cotton might be spilled. Therefore the GMO Panel is of the opinion that the likelihood of the establishment and spread of LLCotton25 is very low and that unintended environmental effects due to this GM cotton will be no different from that of conventional cotton varieties. Furthermore the scope of the monitoring plan provided by the applicant is in line with the intended uses of LLCotton25 since this does not include cultivation.

The GMO Panel is aware that, due to the physical characteristics of cotton seeds and methods of transportation, accidental spillage is unavoidable. Therefore the 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.

CONCLUSIONS AND RECOMMENDATIONS

The GMO Panel was requested to carry out a scientific risk assessment of the LLCotton25 for food and feed uses, import and processing.

LLCotton25 has been modified to express the *bar* gene providing tolerance to glufosinate-containing herbicides. The GMO Panel has evaluated the molecular analysis of the GMO and recognised that only the intended DNA fragment has been integrated at a single locus. From the sequence data provided by the applicant there is no reason to assume that the DNA regions transferred code for toxic and/or allergenic products.

Comparative analysis has shown that the LLCotton25 is compositionally and agronomically equivalent to conventional cotton lines, except for the introduced transgenic trait. The risk assessment included an analysis of data from appropriate animal feeding studies. The GMO Panel concluded that the LLCotton25 is as safe as its non GM counterparts and that the overall allergenicity of the whole plant is not changed.

The application EFSA-GMO-NL-2005-13 concerns import, processing and food/feed uses. There is therefore no requirement for scientific information on possible environmental effects associated with the cultivation of LLCotton25. However the GMO Panel is aware that, due to the physical characteristics of cotton seeds and methods of transportation, accidental spillage is unavoidable. Therefore the 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 GMO Panel considers that information available for LLCotton25 addresses the outstanding questions raised by the Member States and considers it unlikely that LLCotton25 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 Dutch Competent Authority (VROM), dated 3 March 2005, concerning a request for placing on the market of glufosinate-ammonium tolerant cotton LLCotton25 in accordance with Regulation (EC) 1829/2003, submitted by Bayer Crop Science (ref. 050303-BG01).

2. Letter from EFSA to applicant, dated 14 July 2005, with request for clarifications/additional information (ref. SR/SM/sp (2005) 933).

3. Letter from the applicant, dated 5 August 2005, providing EFSA with an updated version of the application EFSA-GMO-NL-2005-13 submitted by Bayer Crop Science under Regulation (EC) 1829/2003:

   Part I – Technical dossier
   Part II – Summary
   Part III – Cartagena Protocol
   Part IV – Labelling and Unique Identifier
   Part V – Samples and Detection
   Part VI – Additional information for GMOs

4. Letter from EFSA to applicant, dated 2nd September 2005, delivering the 'Statement of Validity' for application EFSA-GMO-NL-2005-13, LLCotton25 submitted by Bayer Crop Science under Regulation (EC) 1829/2003 (ref. SR/SM/sp (2005) 1110).

5. Letter from EFSA to applicant, dated 15 September 2005, with request for additional information on detection method/reference material (ref. SR/KL/jq (2005) 1154).

6. Letter from EFSA to applicant, dated 3 November 2005, regarding additional data received from the applicant and the time-schedule for application EFSA-GMO-NL-2005-13 (ref. SR/KL/cz (2005) 1326).

7. Letter from EFSA to applicant, dated 26 January 2006, with request for additional information (ref. SR/SM/cz (2006) 1336033).

8. Letter from applicant to EFSA, dated 17 May 2006, providing additional information upon EFSA request.

9. Letter from EFSA to applicant, dated 23 October 2006, with respect to the time-schedule for application EFSA-GMO-NL-2005-13 (ref. SR/SM/jq (2006) 1797821).

10. Letter from EFSA to applicant, dated 27 October 2006, with request for additional information on detection method/reference material (ref. SR/SM/jq (2006) 1806662).

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