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SCIENTIFIC OPINION

Scientific Opinion on application EFSA-GMO-NL-2011-97 for the placing on the market of insect-resistant and herbicide-tolerant genetically modified cotton T304-40 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience AG[1]

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

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This scientific output, published on 29 July 2013, replaces the earlier version published on 20 June 2013[4].

ABSTRACT

Cotton T304-40 contains a single insert consisting of the *cry1Ab* and the *bar* expression cassettes, providing insect resistance and herbicide tolerance, respectively. Bioinformatic analyses and genetic stability studies did not raise safety issues. Levels of the Cry1Ab and PAT proteins in cotton T304-40 have been sufficiently analysed. No biologically relevant differences were identified in the compositional analysis when the seed of T304-40 was compared with its conventional counterpart and non-GM cotton varieties. The safety assessment identified no concerns regarding the potential toxicity and allergenicity of the newly introduced Cry1Ab and PAT proteins. Based on the information available, there is no evidence that the genetic modification might significantly change the overall allergenicity of cotton T304-40. Nutritional equivalence of cotton T304-40 to its conventional counterparts was indicated by compositional data. The EFSA GMO Panel concludes that cotton T304-40 is as safe and nutritious as its conventional counterpart and that it is unlikely that the overall allergenicity of the whole plant is changed. There are no indications of an increased likelihood of establishment and spread of feral cotton plants. Considering its intended uses as food and feed, interactions with the biotic and abiotic environment were not considered to be an issue. Risks associated with an unlikely but theoretically possible horizontal gene transfer from cotton T304-40 to bacteria have not been identified. The monitoring plan and reporting intervals are in line with the intended uses of cotton T304-40. The EFSA GMO Panel considers that the information available for cotton T304-40 addresses the scientific comments raised by the Member States and states that cotton T304-40, as described in the application, is as safe as its conventional counterpart with

[1] On request from the Competent Authority of the Netherlands for application (EFSA-GMO-NL-2011-97) submitted by Bayer CropScience AG, Question No EFSA-Q-2011-00312, adopted on 30 May 2013.

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[3] Acknowledgement: The Panel wishes to thank the members of the Standing Working Groups on Molecular Characterisation, Food and Feed Safety Assessment and Environmental Risk Assessment on GMO applications, including Christer Andersson, Thomas Frenzel, Marco Nuti and Jean-Michel Wal for the preparatory work on this scientific opinion, and the EFSA staff members, Zoltán Divéki, Christina Ehlert, Yi Liu and Sylvie Mestdagh for the support provided to this scientific opinion.

[4] Editorial changes to the suggested citation were made. The changes do not affect the overall conclusions of the scientific opinion. Number of Key words was reduced to 7. Editorial changes to the EFSA Journal references were made.

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respect to potential effects on human and animal health and the environment in the context of its intended uses as proposed by the applicant.

KEY WORDS

GMO, cotton T304-40, Regulation (EC) No 1829/2003, Cry1Ab, PAT, food and feed safety and environment, import and processing

SUMMARY

Following the submission of an application (Reference EFSA-GMO-NL-2011-97) under Regulation (EC) No 1829/2003[5] from Bayer CropScience AG, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) was asked to deliver a scientific opinion on the safety of the insect-resistant and herbicide-tolerant genetically modified (GM) cotton T304-40 (Unique Identifier BCS-GHØØ4-7) for import and processing for food and feed uses. Cotton T304-40 was developed to provide resistance to certain lepidopteran pests and tolerance to glufosinate ammonium-based herbicides.

In delivering its scientific opinion, the EFSA GMO Panel considered the application EFSA-GMO-NL-2011-97, additional information provided by the applicant (Bayer CropScience AG) and the scientific comments submitted by the Member States. The scope of application EFSA-GMO-NL-2011-97 is for food and feed uses, import and processing of cotton T304-40 and all derived products, but excludes cultivation in the European Union (EU).

The EFSA GMO Panel evaluated cotton T304-40 with reference to the intended uses and appropriate principles described in the guidance documents of the Scientific Panel on Genetically Modified Organisms for the risk assessment of food and feed derived from genetically modified plants (EFSA, 2006a, 2011a). The scientific risk assessment evaluation included molecular characterisation of the inserted DNA and expression of target proteins. A comparative analysis of agronomic traits and composition was undertaken, and the safety of the new proteins, as individual proteins and in combination, the changed levels of natural constituents and the whole food/feed were evaluated with respect to potential toxicity, allergenicity and nutritional quality. Evaluations of environmental impacts and the post-market environmental monitoring plan were undertaken.

Cotton T304-40 has been genetically modified to express the proteins Cry1Ab and phosphinothricin acetyltransferase (PAT). Cry1Ab is an insecticidal protein deriving from *Bacillus thuringiensis* strain *berliner* 1715. PAT is encoded by the *bar* gene deriving from *Streptomyces hygroscopicus*. PAT confers tolerance to glufosinate ammonium-based herbicides.

The molecular characterisation data establish that the GM cotton T304-40 contains a single insert consisting of copies of the *cry1Ab* and the *bar* expression cassettes. No other parts of the plasmid used for transformation are present in cotton T304-40. Bioinformatic analyses and genetic stability studies did not raise safety issues. The levels of the Cry1Ab and PAT proteins in cotton T304-40 have been sufficiently analysed.

Based on the results of compositional analysis of samples from a representative range of environments and seasons, the EFSA GMO Panel concludes that no biologically relevant differences were identified in the compositional characteristics of seeds produced by cotton T304-40 compared with its conventional counterpart, Coker 315, and that its composition falls within the range of estimated natural variation, except for the expression of the Cry1Ab and PAT. In addition, results from field trials did not show indications of unexpected changes in agronomic performance and phenotypic characteristics.

The EFSA GMO Panel has previously evaluated the safety of the Cry1Ab and PAT proteins in the context of several applications for the placing on the EU market of GM crops expressing these proteins, and no concerns were identified. The results of the updated bioinformatic analysis provided in the context of this application do not change these conclusions. In the context of this application the applicant has provided an acute toxicity of Cry1Ab in mice and resistance to degradation by proteolytic enzymes studies of Cry1Ab and PAT proteins associated with the event T304-40. The

[5] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 1–23.

Cry1Ab protein induced no adverse effects in acute oral toxicity study in rodents. Both proteins were rapidly degraded solutions containing pepsin and inactivated during heat treatments.

The safety assessment identified no concerns regarding the potential toxicity and allergenicity of the newly introduced Cry1Ab and PAT proteins. Based on the information available, there is no evidence that the genetic modification might significantly change the overall allergenicity of cotton T304-40. Nutritional equivalence of cotton T304-40 to its conventional counterparts was indicated by compositional data. The EFSA GMO Panel concludes that cotton T304-40 is as safe and nutritious as its conventional counterpart and that it is unlikely that the overall allergenicity of the whole plant is changed.

The application EFSA-GMO-NL-2011-97 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 T304-40 in Europe. There are no indications of an increased likelihood of establishment and spread of feral cotton plants in case of accidental release into the environment of viable cotton T304-40 seeds during transport and/or processing. Considering its intended uses as food and feed, interactions with the biotic and abiotic environment were not considered to be an issue. Risks associated with an unlikely but theoretically possible horizontal gene transfer from cotton T304-40 to bacteria have not been identified. The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of cotton T304-40. Furthermore, the EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the post-market environmental monitoring plan.

In conclusion, the EFSA GMO Panel considers that the information available for cotton T304-40 addresses the scientific issues indicated by the guidance document of the EFSA GMO Panel and the scientific comments raised by the Member States, and that cotton T304-40 is as safe as its comparator with respect to potential effects on human and animal health or the environment in the context of its intended uses.

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BACKGROUND

On 7 April 2011, EFSA received from the Competent Authority of the Netherlands an application (Reference EFSA-GMO-NL-2011-97), for authorisation of insect-resistant and herbicide-tolerant GM cotton T304-40 (Unique Identifier BCS-GHØØ4-7), submitted by Bayer CropScience AG within the framework of Regulation (EC) No 1829/2003 on genetically modified food and feed for food and feed uses, import and processing.

After receiving the application EFSA-GMO-NL-2011-97 and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed the Member States as well as the European Commission and made the summary of the dossier publicly available on the EFSA website. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of Regulation (EC) No 1829/2003. On 29 August 2011 and 3 October 2011, the applicant provided EFSA with additional information requested under completeness check (requested on 26 May 2011 and 12 September 2011, respectively). On 24 October 2011, EFSA declared the application as 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 the Member States, including the national Competent Authorities within the meaning of Regulation (EC) No 1829/2003 and Directive 2001/18/EC[6] following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. The Member State bodies had three months after the date of receipt of the valid application (until 24 January 2012) within which to make their opinion known.

The EFSA GMO Panel carried out a scientific assessment of genetically modified (GM) cotton T304-40 in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003, taking into account the appropriate principles described in the guidance documents of the Scientific Panel on Genetically Modified Organisms for the risk assessment of food and feed derived from genetically modified plants (EFSA, 2006a, 2011a). In addition, the scientific comments of Member States, the additional information provided by the applicant and relevant scientific publications were taken into consideration.

On 5 December 2011 and on 23 April 2012 the GMO Panel asked for additional data on cotton T304-40 (application EFSA-GMO-NL-2011-97). The applicant provided the requested information on 22 March 2012 and on 6 June 2012, respectively. In addition, on 2 May 2013, the applicant provided additional information spontaneously. After receipt and assessment of the full data package, the GMO Panel finalised its risk assessment of cotton T304-40.

In giving its opinion on GM cotton T304-40 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).

[6] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L106, 1–39.

TERMS OF REFERENCE

The GMO Panel was requested to carry out a scientific assessment of the GM cotton T304-40 (Unique Identifier BCS-GHØØ4-7) 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/environments and/or geographical areas should be indicated in accordance with Articles 6(5)(e) and 18(5)e of Regulation (EC) No 1829/2003.

The EFSA GMO Panel was not requested to give an opinion on information required under Annex II to the Cartagena Protocol, nor on the 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 GMO risk management.

ASSESSMENT

1. Introduction

Genetically modified (GM) cotton T304-40 (Unique Identifier BCS-GHØØ4-7) was assessed with reference to its intended uses, taking account of the appropriate principles described in the guidance documents of the Scientific Panel on Genetically Modified Organisms for the risk assessment of food and feed derived from genetically modified plants (EFSA, 2006a, 2011a).

The genetic modification in cotton T304-40 results in the expression of the PAT and Cry1Ab proteins, which confer tolerance to glufosinate ammonium-based herbicides and resistance to certain lepidopteran insects, respectively. The genetic modification in cotton T304-40 is intended to improve agronomic performance only and it is not intended to influence the nutritional properties, processing characteristics and overall use of cotton as a crop.

The scope of application EFSA-GMO-NL-2011-97 is for food and feed use, import and processing of cotton T304-40 within the EU. Thus, cotton T304-40 would be used for the production of cotton products as any commercial cotton variety. Likely uses of cotton T304-40 include the production of refined oil from seeds, production of cellulose from linters as food or food ingredients and use of cotton seed meal and hulls in animal feed.

2. Issues raised by Member States

The scientific comments raised by Member States are addressed in Annex G of the EFSA overall opinion and have been considered throughout this EFSA GMO Panel scientific opinion.

3. Molecular characterisation

3.1. Evaluation of relevant scientific data

3.1.1. Transformation process and vector constructs

Cotton T304-40 was developed by *Agrobacterium tumefaciens*-mediated transformation. Cotyledon explants of *Gossypium hirsutum* variety Coker 315 were co-cultured with a disarmed *A. tumefaciens* strain containing the vector pTDL008.[7]

The pTDL008 vector includes one T-DNA, which contains two expression cassettes between the right and left borders.[8]

The *cry1Ab* cassette confers the insect resistance. It consists of the following genetic elements: a duplicated promoter derived from *Subterranean clover stunt virus* genome segment 7 (Ps7s7); leader sequence of the tapetum-specific E1 gene from *Oryza sativa* (5′*e1*); a synthetic sequence derived from the *cry1Ab* gene of *Bacillus thuringiensis* strain *berliner* 1715, which encodes the N-terminal part of the proprotein sufficient to produce a functional toxin; 3′ untranslated region of the NADP-malic enzyme encoding gene from *Flaveria bidentis* (3′ *me*1).

The *bar* cassette confers tolerance to glufosinate ammonium-based herbicides. It contains the 35S promoter from *Cauliflower mosaic virus* (P35S3); the coding sequence of the phosphinothricin acetyltransferase gene from *Streptomyces hygroscopicus* (*bar*); and the 3′ untranslated region of the nopaline synthase gene from *A. tumefaciens* (3′ *nos*).

The vector backbone contained elements necessary for the maintenance and selection of the plasmid in bacteria: *ori* ColE1, the origin of replication from plasmid pBR322, required for the maintenance of

[7] Technical Dossier/Section C1
[8] Technical Dossier/Section C2

pTDL008 in *Escherichia coli*; *ori* pVS1, the origin of replication from the *Pseudomonas* plasmid pVS1, for the maintenance of the plasmid vector in *Agrobacterium*; a fragment of the bacterial *npt*I gene; the bacterial *aad*A gene, conferring resistance to streptomycin; and residual sequences of pTiAch5 flanking the left and right border repeats.

3.1.2. Transgene constructs in the genetically modified plant

Molecular characterisation of cotton event T304-40 was performed by Southern analysis and sequencing, to determine copy number, size and organisation of the inserted sequences and to confirm the absence of plasmid backbone sequence.[9] The approach used was acceptable in terms of both coverage and sensitivity.

Southern blot hybridisation data demonstrated that the inserted transgenic sequence in cotton event T304-40 consists of one nearly complete copy of the T-DNA, flanked by an inverted incomplete copy of the *crylAb* gene cassette and one additional 3′ *me*1 terminator and a partial copy of the *bar* expression cassette, containing a truncated 3′ *nos* terminator. The organisation of the insert was confirmed by the hybridisation signals generated with a set of 10 different restriction enzymes in combination with seven distinct probes. The absence of vector backbone was demonstrated by Southern analysis, using seven overlapping probes.[10]

Results of the sequence analysis of the insert in cotton T304-40 and its 5′ and 3′ flanking regions were in line with those indicated by the Southern analyses. A comparison of the pre-insertion locus in wild-type cotton with T304-40 indicated that the pre-insertion locus was preserved except for the deletion of 32 bp. Alignment of the insert sequence with the corresponding regions of pTDL008 indicated that they are identical except for a single nucleotide change in the copy of the non-coding 3′ *me*1 terminator region located close to the 5′ junction.[11] Bioinformatic analyses of the genomic sequences flanking the insert and the pre-insertion site were carried out to assess any potential interruption of known cotton genes.[12] BLASTN searches were performed against plant EST (Expressed Sequence Tag) database and non-redundant nucleotide database and BLASTX searches against non-redundant amino acid database, and the results did not indicate the interruption of any known gene in cotton T304-40. The results also confirmed that the insert is located in the nuclear genome.

In order to assess whether the open reading frames (ORFs) present within the insert and spanning the junction sites raise any safety issue, their putative translation products were compared with appropriate databases for similarities to known allergens and toxins by using suitable algorithms.[13] None of the ORF translation products identified spanning the junction sites in cotton T304-40 showed biologically significant sequence similarities with known toxins or allergens. Epitope homology searches showed identity between a contiguous block of 8 amino acids of an ORF present within the insert (ORF.100) and two known allergens belonging to the glycinin G5 subgroup. Apart from this epitope match, ORF.100 does not show above-threshold similarity to any allergens as defined by Codex Alimentarius (2009) and the EFSA scientific opinion on assessment of allergenicity of GM plants (EFSA, 2010a). Although ORF.100 does not have a start codon, the applicant performed northern analysis on RNA extracted from cotton T304-40.[14] No mRNA containing ORF.100 as an exon could be detected, which provides additional evidence that the expression of ORF.100 is unlikely.

3.1.3. Information on the expression of the insert

Cotton T304-40 expresses two new proteins, Cry1Ab and PAT. Protein levels were analysed in greenhouse-grown plants by enzyme-linked immunosorbent assay (ELISA) in roots, stems, leaves,

[9] Technical Dossier/Section D2
[10] Technical Dossier/Section D2 (a)
[11] Technical Dossier/Section D2 (c)
[12] Technical Dossier/Section D2 (b)
[13] Technical Dossier/Section D2 (b)
[14] Additional information, June 2012

squares, apices, bolls, pollen, nectar, flowers and seeds. Considering the scope of the application, the Cry1Ab and PAT protein levels in seeds are considered most relevant. The levels of the newly expressed proteins were determined in seeds harvested from field trials performed in the USA (2007) and in Spain (2008) at locations representing typical cotton-growing regions.[15] Each site contained both glufosinate ammonium-treated and untreated plots. In 2007 and 2008, data were collected from eight and six sites, respectively. The mean Cry1Ab level for seed across all USA sites was 0.562 µg/g fresh weight (fw) (range 0.527–1.57 µg/g fw); the corresponding value for the European trials was 2.63 µg/g fw (range 1.77–3.63 µg/g fw). The mean PAT level for seeds across all USA sites was 97.7 µg/g fw (range 67.5–138 µg/g fw); the corresponding value for the European trials was 146 µg/g fw (range 97.8–222 µg/g fw). The results showed that the means and ranges of Cry1Ab and PAT proteins in cotton T304-40 grown in 2007 in the USA were generally lower than those observed in samples collected from cotton T304-40 grown in 2008 in Spain. Variations in protein levels such as those observed are not unexpected in different field trials, and do not pose a safety concern *per se*. Comparison of the herbicide-treated and untreated plots indicated that glufosinate ammonium treatment had no significant effect on the levels of the newly expressed proteins in seeds.

The possible presence of potential fusion proteins in cotton T304-40 was analysed because of the truncated terminators in the insert. Although northern analysis showed the presence of read-through transcripts of the *cry1Ab* gene, western analysis did not indicate fusion proteins. Prior bioinformatic analysis of the DNA sequences corresponding to the read-through transcripts did not show similarity to known toxins and allergens. Northern analysis did not reveal any read-through transcripts from the *bar* gene.

3.1.4. Inheritance and stability of inserted DNA

Genetic stability of the T304-40 insert was studied by Southern analysis. The tested samples represented four generations and a variety of genetic backgrounds and cultivation environments. The restriction enzyme–probe combinations used were sufficient to conclude that a single-copy insert together with its flanking regions was retained over several generations, indicating stability.[16]

Supporting evidence for the phenotypic stability was obtained by segregation analysis of samples from two generations of plants with three different backgrounds. The results supported the presence of a single genetic locus, showing Mendelian segregation.

3.2. Conclusion

The molecular characterisation data establish that cotton T304-40 contains a single insertion locus consisting of partial copies of the *cry1Ab* and *bar* expression cassettes. Up-to-date bioinformatic analyses of the ORFs spanning the junction sites within the insert or between the insert and genomic DNA did not identify hazards. The stability of the inserted DNA and the introduced traits, namely the insect resistance and the herbicide tolerance, were confirmed over several generations. The potential impacts of the Cry1Ab and PAT protein levels, quantified in field trials carried out in the USA and in Spain, are assessed in the food/feed and environment sections.

4. Comparative analysis

4.1. Evaluation of the relevant scientific data

The GMO Panel has considered the data on the compositional, agronomic and phenotypic characteristics of cotton T304-40 and its comparators as provided in the dossier and summarised below.

[15] Technical Dossier/Section D3 (a), (b) and (d)
[16] Technical Dossier/Section D5

4.1.1. Choice of comparator and production of material for the comparative analysis[17]

Field trials for the analysis of composition and agronomic and phenotypic characteristics of cotton T304-40 and its conventional counterpart were carried out at various locations in the USA during one season (2007)[18] and the EU during two seasons (2007 and 2008).[19] The conventional counterpart in these field trials was Coker 315, which has a similar genetic background to cotton T304-40.

The eight field trials in the USA in 2007 were performed in Louisiana (two sites), Mississippi (two sites) and Texas (four sites), and included cotton T304-40 treated twice with glufosinate ammonium-based herbicides during the growing season, cotton T304-40 treated with maintenance herbicides/pesticides (but not with glufosinate ammonium) and plots with the conventional counterpart treated with maintenance herbicides/pesticides. In each location the field trial had a complete randomised block design with three replications of each treatment. Fuzzy seed samples were harvested from each plot and ginned before further dispatch and handling for compositional analysis.[20]

In the EU field trials were carried out in Spain in 2007 and 2008 in the regions of Catalonia[21] (12 sites in 2007, eight sites in 2008) and of south Andalusia[22] (six sites in 2008). In the Catalonian locations, a complete randomised block design with three replications and three treatments was conducted: cotton T304-40 treated twice with glufosinate ammonium-based herbicides; cotton T304-40 treated with maintenance herbicides; and the conventional counterpart treated with maintenance herbicides. In addition, two commercial reference varieties (Celia, Flora) were planted in each location, in single non-replicated plots in both years. In Andalusia, the same experimental design was followed, except that the commercial varieties included were different (Alexandro, Crema 111, Hermes) and a total of six field trials were planted with five replicates for each cotton line (T304-40 treated with glufosinate ammonium-based herbicide and T304-40, Coker 315 and commercial lines untreated with glufosinate ammonium-based herbicide). After harvest, fuzzy seed were ginned locally before further handling. Compositional analysis was performed on seed from eight Catalonian locations in 2007, and six Andalusian and two selected Catalonian locations in 2008.

During both years of cultivation and at all field trial sites, various studies were carried out for the analysis of agronomic and phenotypic traits.

4.1.2. Compositional analysis[23]

The compounds analysed in cotton seeds followed the recommendations of OECD (2009). Harvested cotton seeds were analysed for proximates (protein, fat, ash, moisture and carbohydrate by calculation), fibre fractions (acid detergent fibre (ADF), neutral detergent fibre (NDF) and crude fibre (CF)), amino acids, fatty acids, vitamin E, minerals (calcium, phosphorus, potassium, magnesium, iron and zinc) and anti-nutrients (gossypol and cyclopropenoid fatty acids).

The statistical analysis was an overall-site analysis of variance (ANOVA) to compare the conventional counterpart sprayed with required maintenance pesticides and cotton T304-40 sprayed with either glufosinate ammonium or required maintenance herbicides only. The ANOVA results indicated a statistically significant interaction between treatment and site for many parameters. For these parameters, the applicant performed *t*-tests comparing cotton T304-40 (sprayed or non-sprayed with glufosinate ammonium) with its conventional counterpart for each site separately.

The statistical analysis of the compositional data of cotton seeds harvested from field trials in the USA in 2007 identified 16 statistically significant differences between cotton T304-40 sprayed with

[17] Technical dossier/Section D7.2
[18] Technical dossier/Mackie (2008)
[19] Technical dossier/Villagran (2008)
[20] Technical dossier/Oberdörfer (2009a)
[21] Technical dossier/Villagran (2008, 2009b)
[22] Technical dossier/Villagran (2009a)
[23] Technical dossier/D7.3

required maintenance pesticides and the conventional counterpart Coker 315 and 18 statistically significant differences between cotton T304-40 treated with glufosinate ammonium and the conventional counterpart Coker 315 sprayed with maintenance pesticides (untreated) (Appendix 1, Table 1).

The applicant initially performed a joint analysis of the compositional data from cotton seeds harvested from the EU field trials in 2007 and 2008. Upon request from the EFSA GMO Panel, the applicant analysed the data from the two years separately.

The analysis of data from the EU in 2007 identified 17 statistically significant differences between cotton T304-40 sprayed with required maintenance pesticides (untreated) and the conventional counterpart Coker 315, and 13 statistically significant differences between cotton T304-40 treated with glufosinate ammonium and the conventional counterpart Coker 315 (Appendix 1, Table 2).

The analysis of the EU 2008 field trial identified 18 statistically significant differences between T304-40 sprayed with required maintenance pesticides and the conventional counterpart Coker 315 and 19 statistically significant differences between T304-40 treated with glufosinate ammonium and the conventional counterpart Coker 315 (Appendix 1, Table 3).

The level of calcium, zinc, linoleic acid, palmitic acid and stearic acid showed statistically significant differences in cotton T304-40 and Coker 315 over all three seasons of field trials and both treatment regimes with herbicides. However, the endpoint values for calcium, zinc, palmitic acid and stearic acid were within the limits of the reference varieties used in the European field trials in 2007 and 2008. The values for linoleic acid in both T304-40 and Coker 315 were higher than the range in values of the reference varieties in the European field trials in 2007, but within the range set by the reference varieties in the field trials in 2008.

Having considered the total set of compositional data supplied and the observed compositional differences between cotton T304-40 and its conventional counterpart Coker 315, the nature and magnitude of the differences and biological variation, the EFSA GMO Panel concludes that no biologically relevant differences were identified in the compositional characteristics of seeds produced by cotton T304-40 compared with its conventional counterpart Coker 315, and that its composition falls within the range of estimated natural variation, except for the expression of the Cry1Ab and PAT proteins.

4.1.3. Agronomic and phenotypic characteristics[24]

Based on data collected at eight field trial sites in Spain in 2007 and 2008, the applicant performed a comparative assessment of the phenotypic and agronomic characteristics of cotton T304-40 and its conventional counterpart (Coker 315). The phenotypic and agronomic characteristics evaluated were plant height at maturity, number of developed nodes, presence and development of the fruiting organ at the first position on each node, seed cotton yield, stand count, fibre yield, number of seed per 10 bolls, weight of fuzzy seed per 10 bolls, number of days to first flower, number of days to first boll, susceptibility to fungi, first fruiting branch, number of living insect larvae on immature blooms, flowers and bolls (analysed at two dates), number of immature blooms, flowers and bolls damaged by insects (analysed at two dates), fibre fineness (micronaire), fibre length, fibre length uniformity, short fibre index, fibre strength and fibre elongation.

The applicant analysed the data using ANOVA, which identified statistically significant differences between cotton T304-40 and its conventional counterpart for the endpoints stand count, first fruiting branch, fibre yield, number of seeds per 10 bolls, weight of fuzzy seed per 10 bolls, number of days to first boll, number of flowers damaged by insects at date 1, number of bolls damaged by insects at date

[24] Technical dossier/D7.4

2, number of living larvae on bolls at both dates, fibre length uniformity, short fibre index and fibre strength.

The differences for insect damage and presence of insect larvae were expected, as cotton T304-40 expresses the insecticidal Cry1Ab protein. All the other statistically significant differences were within the natural variation of reference varieties. The differences in fibre parameter such as length, strength and micronaire did not lead to a different classification of the fibre quality. Based on the data presented by the applicant, the agronomic and phenotypic characteristics of insect-resistant and glufosinate ammonium-tolerant cotton event T304-40 are comparable to those of its conventional counterpart, Coker 315, and to the commercial non-GM cotton reference varieties grown in the same field trials.

4.2. Conclusion

Based on the information available, the EFSA GMO Panel concludes that no biologically relevant differences were identified in the composition, agronomic and phenotypic characteristics of plants and seeds obtained from cotton T304-40 that would require further assessment.

5. Food/feed safety assessment

5.1. Evaluation of relevant scientific data

5.1.1. Effects of processing[25]

The newly expressed proteins (Cry1Ab and PAT) were detectable in delinted seeds, cotton seed meal and hulls but non-detectable in crude and food grade oil of the GM cotton T304-40 in materials harvested from the USA field trial in 2009; they were present in toasted meal at very low levels. PAT was present in cotton seed and kernels; undetectable in refined oil; and either present at low levels or non-detectable in hulls and toasted meal, in all three years.[26] Considering the toxicological profile and allergenic properties (see sections 5.1.2 and 5.1.3), the presence of Cry1Ab and PAT protein in derived products obtained through seed processing would not raise safety concern.

Since no biologically relevant differences were identified in the composition, agronomic and phenotypic characteristics of plants and seeds obtained from cotton T304-40, the effect of processing on cotton T304-40 is not expected to be different from that on conventional cotton.

5.1.2. Toxicology[27]

5.1.2.1. Protein used for safety assessment

Given the low levels of expression of the proteins Cry1Ab and PAT in tissues of cotton T304-40 (below the level of quantification in seed, see section 3.1.3) and the difficulty of producing sufficient amounts for safety testing, recombinant Cry1Ab and PAT proteins produced in *E. coli* were used for safety testing. The structural and functional equivalence of bacterially produced Cry1Ab and PAT proteins to those expressed in cotton T304-40 was investigated with SDS-PAGE, western blotting, glycosylation analysis, N-terminal sequence analysis and mass spectrometry. These studies indicated that there were small differences between the plant-derived and microbial Cry1Ab proteins in both the N- and C-termini. However, there were no differences in insecticidal properties (Cry1Ab) or enzymatic activity (PAT) between the plant-derived and microbial proteins.[28] Therefore, the GMO Panel accepts the use of the Cry1Ab and PAT proteins expressed in bacteria for the safety testing of the Cry1Ab and PAT proteins present in cotton T304-40.

[25] Technical dossier/Section D7.1.6
[26] Technical dossier/Mackie (2009)
[27] Technical dossier/Section D7.2
[28] Technical dossier/Section D7.8.1.iv.

5.1.2.2. Toxicological assessment of newly expressed proteins in cotton T304-40

The EFSA GMO Panel has previously evaluated the safety of the PAT protein in the context of several applications for the placing on the EU market of GM crops expressing PAT, and no concerns were identified (e.g. EFSA, 2005a, b, 2006b, 2007a, b, c, 2008, 2011c, 2012a).

There is a considerable body of knowledge on Cry1Ab, including *in vivo*, *in vitro* and *in silico* studies, which does not indicate safety concerns (EFSA, 2005b, 2009b, c, 2012b, c). The results of the updated bioinformatic analysis provided in the context of this application do not change these conclusions.[29] In the context of this application the applicant has provided an acute toxicity of Cry1Ab in mice and *in vitro* digestibility studies of Cry1Ab and PAT proteins newly expressed by cotton T304-40.

(a) Acute oral toxicity testing

The Cry1Ab protein was administrated by oral gavage at a dose of 2 000 mg/kg body weight to a single group of five female mice. One animal died for reasons considered unrelated to treatment. No treatment-related adverse effects were observed.[30]

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

(b) Bioinfomatic studies[31]

Bioinformatic analyses of the amino acid sequences of the Cry1Ab and PAT proteins expressed in cotton T304-40 revealed no significant similarities to known toxic proteins.

(c) Resistance to degradation by proteolytic enzymes[32]

The resistance to degradation by pepsin of the Cry1Ab protein was measured in solutions at pH 1.2. The integrity of the test protein in samples of the incubation mixture taken at various time points was analysed by gel electrophoresis followed by protein staining. No Cry1Ab protein was detected within two minutes of incubation.

The resistance to degradation by pancreatin of the Cry1Ab protein was also assessed in solutions at pH 7.5. Stable fragments of different molecular weight of the Cry1Ab protein were observed at different time points. The EFSA GMO Panel notes that this study is not required by either EFSA guidance documents (EFSA, 2006a, 2011a) or Codex Alimentarius (2009).

Published data showed that a bacterially produced analogue of the PAT protein encoded by the *bar* gene is degraded by pepsin within 30 seconds and by pancreatin within five minutes (Hérouet et al., 2005). As previously mentioned, the resistance to degradation by pancreatin study is not required by international guidelines (EFSA, 2006a, 2011a; Codex Alimentarius, 2009).

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

(a) Sub-chronic toxicity study[33]

In a sub-chronic, 13-week feeding study, groups of 10 male and 10 female rats (strain Wistar Rj:WI (IOPS HAN)) received diets containing toasted cotton T304-40 seed meal at 5 % (supplemented with

[29] Technical dossier/Section D7.8, Additional information, May 2013
[30] Technical dossier/Section D7.8.1
[31] Technical dossier/Section D7.8, Additional information, May 2013
[32] Technical dossier/Section D7.8.1.i
[33] Technical dossier/Section D7.8.1.iii

5 % Coker 315 cotton) and 10 % inclusion level, the conventional counterpart Coker 315 (inclusion level 10 %) or the commercial non-GM control FM958.

Animals were housed in cages with five rats of the same sex per cage, but the data analysis considered the individual animal as the experimental unit, ignoring a possible bias due to cage interactions. As the cage should be considered the experimental unit and because of the low number of experimental units per treatment (two per sex), a statistical analysis of the data is not possible.

(b) Chicken feeding study

Zootechnical parameters of chicken for fattening fed diets (starter first week, grower second and third week, finisher until end) containing 10 % toasted cotton seed meal each prepared from the GM cotton T304-40, the conventional counterpart (Coker 315) and one commercial non-GM cotton variety (FM958), respectively, for 42 days were compared.[34] A total of 420 one-day-old chicken (ROSS 308, half male and half female) broiler chicks were randomly assigned to one of the three treatments, each treatment consisting of 14 replicate pens (seven pens per sex, 10 birds per pen). The diets containing approximately 10 % of toasted cotton seed meal were formulated to meet poultry nutrient requirements under typical local industry husbandry and to be isonitrogenous, isocaloric and balanced for sulphur and limiting amino acids (analytically confirmed). Diets with the cotton T304-40 seed meal, but not the control diets or the reference diets, contained the *cry1Ab* gene.

Effects on health, survival, live weight, total weight gain, feed consumption, feed to gain ratio, marketable carcass weight and muscle tissue weight and yield (breast, thigh, leg, wing) and abdominal fat pad weight were compared among groups. Body weight, weight gain, feed intake and feed to gain ratio were measured or calculated at weekly intervals until the end of the trial.

Statistical evaluation was carried out by ANOVA using the pen as the experimental unit for survival, feed consumption and feed to gain ratio, and using the individual bird measurements for the other parameters.

Overall mortality/cull was 10.5 % (44 birds). Post-mortem analysis showed ascites for 35 birds, and petechia in the lung or haemorrhages in the lung or respiratory tract for the others. Deaths were not treatment related.

The final body weight was 2.75 kg in the T304-40 group, 2.78 kg in the Coker 315 group and 2.82 kg in the FM958 group. The cumulative feed to gain ratio was 1.83 in the T304-40 group, 1.80 in the Coker 315 group and 1.78 in the FM 958 group. No significant differences were found for final body weight, weight gain, feed intake or feed to gain between the groups. As expected, females had a lower body weight, consumed less feed and showed a higher feed to gain ratio than males. A statistically significant treatment × sex interaction was observed for feed consumption but not for feed to gain ratio. At study termination the subset of 42 randomly selected birds/treatment processed (three birds per cage) for carcass and tissue weights was examined for gross pathology. Two abnormal observations (both in the T304-40 group: one male with ascites, congested intestines and enlarged heart and liver, and one female with pericarditis and perihepatitis) were made at this time. Of the remaining birds, carcass characteristics were not significantly different between groups. There was no significant treatment by sex interaction.

Since all diets were designed to deliver the same nutrition, the expectation was that birds in the three groups would show essentially the same performance characteristics. The results confirmed the nutritional value of the cotton T304-40 seed and the absence of any unintended effects able to impact on growth at the level tested. Having considered the design and outcome of the feeding study with chicken for fattening, the Panel notes that the value of the study is limited by (i) the high mortality observed and (ii) the low dietary inclusion level of cotton seed.

[34] Technical dossier/Section D7.8.4, Study report M-350594-02-1 (2010)

5.1.3. Allergenicity[35]

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 plant.

5.1.3.1. Assessment of allergenicity of the newly expressed protein

A weight-of-evidence approach is followed, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (EFSA, 2006a, 2011a; Codex Alimentarius, 2009).

The *cry1Ab* gene originates from *B. thuringensis* subsp. *kurstaki*, a soil microorganism that is not known to be allergenic. The source of the *bar* gene encoding for the PAT protein is *S. hygroscopicus*, which is also a soil microorganism that is not known to be allergenic.

In the current application (EFSA-GMO-NL-2011-97), updated bioinformatic analyses[36] of the amino acid sequences of the Cry1Ab and PAT proteins using the criterion of 35 % identity in a window of 80 amino acids revealed no significant similarities to known allergens. In addition, the applicant performed analyses[22] searching for matches of eight contiguous identical amino acid sequences between the Cry1Ab and PAT proteins and known allergens, which confirmed the outcome of the previous bioinformatic analyses.

The studies on resistance to degradation of Cry1Ab and PAT proteins by proteolytic enzymes have been described in section 5.1.2.2.

The EFSA GMO Panel has previously evaluated the safety of the Cry1Ab and/or PAT protein in the context of several applications and no concerns on allergenicity were identified (e.g. EFSA, 2005a, b, 2006b, 2007a, b, c, 2008, 2009b, c, 2010b, 2011c, 2012a, b, c).

Based on the available information, the EFSA GMO Panel considers that there are no indications that the newly expressed Cry1Ab and PAT proteins in cotton T304-40 may be allergenic.

5.1.3.2. Assessment of allergenicity of the whole GM plant

According to the guidance documents of the Scientific Panel on Genetically Modified Organisms for the risk assessment of food and feed derived from genetically modified plants (EFSA, 2006a, 2011a), when the plant receiving the introduced gene is known to be allergenic, the applicant should test any potential change in the allergenicity of the whole GM plant by comparing the allergen repertoire with that of its appropriate comparator(s).

Cotton has not been considered to be a common allergenic food[37] and only a few cases of food allergy to cotton seed have been reported (Atkins, 1988; Malanin and Kalimo, 1988; O'Neil and Lehrer, 1989), all of which were to foods with cotton seed flour as the offending ingredient. Cotton seed protein appears to contain allergen(s) of relevant potency. However, the main cotton seed product in human food, industrially processed cotton seed oil, is highly purified and contains negligible levels of proteins. Also in cellulose from cotton seed linters for use as food or food ingredient, the protein level is considered to be very low.

[35] Technical dossier/Section D7.9
[36] Additional information, May 2013
[37] Directive 2007/68/EC of the European Parliament and of the Council of 27 November 2007 amending Annex IIIa to Directive 2000/13/EC of the European Parliament and of the Council as regards certain food ingredients. OJ, L310, 11–14.

In the context of the present application and based on the available information, the EFSA GMO Panel considers that there is no evidence that the genetic modification might significantly change the overall allergenicity of cotton T304-40.

5.1.4. Nutritional assessment of food/feed derived from GM plants

The intended trait of cotton T304-40 is insect resistance and herbicide tolerance, with no intention to alter the nutritional parameters. The outcome of the composition analysis (see section 4.1.2) confirmed the nutritional equivalence of the food and feed products derived from cotton T304-40. The introduction of these products into the food and feed chain is, therefore, expected to have no nutritional impact, as compared with its conventional counterpart and non-GM cotton varieties.

The compositional data indicated nutritional equivalence between the GM cotton T304-40 and commercial non-GM cotton varieties (see section 4.1.2). Owing to limitations in the feeding study in broilers, these results could not be confirmed (see section 5.1.2.3.b). Nevertheless, in accordance with the EFSA guidance document (EFSA, 2006a, 2011a), the EFSA GMO Panel concludes that cotton T304-40 is as nutritious as other cotton varieties commercially available.

5.1.5. Post-market monitoring of GM food/feed

No biologically relevant compositional, agronomic and phenotypic changes were identified in cotton T304-40 when compared with its conventional counterpart and commercial cotton varieties. Furthermore, the overall intake or exposure is not expected to change because of the introduction of cotton T304-40 into the market. The EFSA GMO Panel therefore considers cotton T304-40 to be as safe as its conventional counterpart and that post-market monitoring (EFSA, 2006a, 2011a) of the food/feed derived from cotton T304-40 is not necessary.

5.2. Conclusion

The safety assessment identified no concerns regarding the potential toxicity and allergenicity of the newly introduced Cry1Ab and PAT proteins. Based on the information available, there is no evidence that the genetic modification might significantly change the overall allergenicity of cotton T304-40. Nutritional equivalence of cotton T304-40 to its conventional counterparts was indicated by compositional data. The EFSA GMO Panel concludes that cotton T304-40 is as safe and nutritious as its conventional counterpart and that it is unlikely that the overall allergenicity of the whole plant is changed.

6. Environmental risk assessment and monitoring plan

6.1. Evaluation of relevant scientific data

The scope of application EFSA-GMO-NL-2011-97 is for food and feed uses, import and processing and does not include cultivation. Considering the intended uses of cotton T304-40, the environmental risk assessment is concerned with indirect exposure, mainly through ingestion by animals, and their manure and faeces leading to exposure of gastrointestinal tract and soil microorganisms, and with the accidental release into the environment of viable cotton T304-40 seeds (e.g. during transport and/or processing).

Cotton T304-40 has been developed for tolerance to glufosinate ammonium-based herbicides and protection against certain lepidopteran pests such as cotton bollworm larvae and tobacco budworm larvae. Herbicide tolerance is conferred by the expression of the PAT protein. Insect resistance is achieved by the expression of the *B. thuringiensis*-derived Cry1Ab protein. As the scope of the present application excludes cultivation, environmental concerns in the EU related to the use of glufosinate ammonium-based herbicides on the GM cotton do not apply.

6.1.1. Environmental risk assessment

6.1.1.1. Unintended effects on plant fitness due to the genetic modification[38]

Gossypium hirsutum is highly domesticated crop which has been grown in southern Europe since the nineteenth century, giving rise to feral plants which can occasionally be found in the same area (Todaro, 1917; Davis, 1967). In the EU,[39] cotton is cultivated in Greece and Spain, and there is about 700 hectares in Bulgaria (USDA, 2009). The main cultivated cotton (*G. hirsutum*), which has been present in southern Europe since the nineteenth century, is an annual self-pollinating crop. In the absence of insect pollinators (such as wild bees, honeybees, bumblebees), cotton flowers are self-pollinating, but when these pollinators are present low frequencies of cross-pollination can occur (McGregor, 1959; Moffett and Stith, 1972; Moffett et al., 1975; Van Deynze et al., 2005).

Pollen and seed dispersal by cotton are potential sources of vertical gene flow to cross-compatible wild cotton relatives, other cotton varieties and 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 (120–200 µm), heavy and sticky, wind-mediated dispersal of pollen to other cotton varieties is considered negligible (Vaissiere and Vinson, 1994). In addition, cross-pollination percentages steeply 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, 2011; Zhang et al., 2005; Hofs et al., 2007; Llewellyn et al., 2007; Heuberger et al., 2010).

Seeds are the only survival structures. However, 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). In regions where cotton is widely grown, such as Australia, the risk of GM cotton becoming feral along transportation routes, or a weed on dairy farms where raw cotton seed is used as feed, has been shown to be negligible (Addison et al., 2007). Since the general characteristics of cotton T304-40 are unchanged relative to its conventional counterpart, the inserted insect resistance trait is not likely to provide a selective advantage outside of cultivation in Europe. If accidental spillage and subsequent release into the environment of cotton T304-40 seeds occurs, cotton T304-40 plants would have a selective advantage only under conditions of high infestation by susceptible *Lepidopteran* species or application of glufosinate ammonium-based herbicides which are not commonly used on cultivated cotton or in most areas where the GM cotton might be spilled. Insect resistance against certain lepidopteran pests, such as cotton bollworm (CBW, *Helicoverpa armigera*), pink bollworm (PBW, *Pectinophora gossypiella*) and tobacco budworm (TBW, *Heliothis virescens*) provides a potential advantage in cultivation under infestation conditions, but plant survival is also limited by sensitivity to a range of other environmental factors. It is thus considered very unlikely that cotton T304-40 or its progeny will differ from other cotton varieties in their ability to survive until subsequent seasons or establish feral populations under European environmental conditions.

Field trials with cotton T304-40 were carried out by the applicant across 12 locations in Spain in 2007 and across 14 locations in Spain in 2008, as described in section 4.1.1. In several Catalonian locations (three in 2007, two in 2008) and two Andalusian locations (in 2008), fuzzy seeds of T304-40 and Coker 315 were re-sown after harvest to assess their potential overwintering capacity.

As mentioned above, the combined site analysis of the 2007 and 2008 field data identified statistically significant differences between treated and untreated cotton T304-40 and the conventional counterpart in the first bolls position, plant emergence, seed number, seed weight, days until boll formation, damage to flowers (first observation date) and living larvae in bolls (both observation dates).

[38] Technical Dossier/Section D4 and D7.4
[39] http://epp.eurostat.ec.europa.eu/portal/page/portal/agriculture/data/database

Considering the scope of the application, special attention is paid to those agronomic characteristics which may affect the survival, establishment and fitness of cotton T304-40 seeds which could be accidentally released into the environment: yield, plant height, shattering, germination and dormancy. None of the significant differences observed was indicative of a consistent plant response associated with the trait. The EFSA GMO Panel considers that the small differences observed are unlikely to affect the overall fitness, invasiveness or weediness of the GM cotton.

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 traits introduced by the genetic modification result in increased persistence and invasiveness of a crop species. 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 EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects of the cotton T304-40 in Europe will not be different from that of conventional cotton varieties.

6.1.1.2. Potential for gene transfer[40]

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

(a) Plant-to-bacteria gene transfer

Genomic DNA is a component of many food and feed products derived from cotton. It is well documented that DNA present in food and feed becomes substantially degraded during processing and digestion in the human or animal gastrointestinal tract. However, a low level of exposure of fragments of ingested DNA, including the recombinant fraction of such DNA, to bacteria in the digestive tract of humans, domesticated animals and other environments exposed to the GM plant or plant material is expected.

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

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

Cotton T304-40 contains genetic elements with identity or high similarity to those of bacteria. These are the coding sequence of Cry1Ab, a codon-optimised sequence of *cry1Ab* from *B. thuringiensis*, and the coding sequence of the phosphinothricin acetyltransferase (*bar*) gene of *Streptomyces hygroscopicus*. The flanking regions of the recombinant gene insert contain approximately 20-bp-long sequences of the truncated right and left border of the Ti plasmid of *A. tumefaciens*. Both species, *B.*

[40] Technical Dossier/Section D6

thuringiensis and *S. hygroscopicus*, are not considered to be prevalent in the main receiving environment, i.e. the gastrointestinal tract of humans or animals. Both occur in soil and, in addition, *B. thuringiensis* has been frequently isolated from the guts of insects (Jensen et al., 2003). However, occurrence of the recombinant genes outside their immediate receiving environment in the habitats of both bacterial species cannot be ruled out (Hart et al., 2009) and is therefore also considered here.

On a theoretical basis (i.e. in the absence of experimental evidence of horizontal gene transfer in GM food and feed derived from cotton T304-40 or any other GM plant), it can be assumed that, as an extremely rare event, homologous recombination may occur in the environment between the recombinant *cry1Ab* or *bar* genes and their natural variants as they may occur in *B. thuringiensis* (for *cry1Ab*) and *S. hygroscopicus* (for *bar*) or other bacteria. Such recombination events would replace only natural variants (i.e. substitutive recombination) and are therefore unlikely to provide any new property connected to a selective advantage for the recipient organisms (EFSA, 2009a). Double homologous recombination of the flanking regions with those on Ti plasmids of *A. tumefaciens* would result in gene replacement, by which a *cry1Ab-bar* gene construct would substitute genes for crown gall formation (loss of auxin-, cytokinin- and opine-synthesising genes). However, the current literature suggests a minimal length of approximately 150 bp of homologous DNA to facilitate recombination (De Vries and Wackernagel, 2002). Therefore, the 20-bp flanking regions of the Ti-plasmid can probably not be considered to contribute to a horizontal gene transfer of the recombinant gene to natural strains of *Agrobacterium* with Ti plasmids.

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

The *cry1Ab* and *bar* genes of T304-40 are regulated by promoters of the *Subterranean clover stunt virus* or the *Cauliflower mosaic virus*, respectively. The expression of the *Ps7s7-cry1Ab* and *p35S3-bar* constructs in bacteria is unknown, but generally the expression level of eukaryotic promoters in bacteria is inefficient (Warren et al., 2008).

The EFSA GMO Panel concludes that the *cry1Ab* or *bar* genes from cotton T304-40 may, on a theoretical basis, replace natural variants (substitutive recombination) and are therefore unlikely to provide any new property connected to a selective advantage for the recipient organisms. Considering its intended uses as food and feed and the above assessment, the EFSA GMO Panel has not identified any concern associated with horizontal gene transfer from cotton T304-40 to bacteria.

(b) Plant-to-plant gene transfer

Considering the intended uses of cotton T304-40 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.

The genus *Gossypium* consists of at least four species: *G. arboreum*, *G. barbadense*, *G. herbaceum* and *G. hirsutum*. *G. herbaceum* is reported to be a traditional fibre crop in the eastern Mediterranean area already in the pre-Columbus period (before 1500 AD) (Zohary and Hopf, 2000). In southern Europe, *G. herbaceum* and *G. hirsutum* have been grown since the nineteenth 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 this GM cotton is restricted to cultivated and occasional feral populations.

Insect resistance against certain lepidopteran pests, such as European cotton bollworm (CBW, *Helicoverpa armigera*), pink bollworm (PBW, *Pectinophora gossypiella*) and tobacco budworm

(TBW, *Heliothis virescens*), provides an advantage in cultivation under infestation conditions. Survival of cotton outside cultivation in Europe is mainly limited by a combination of low competitiveness, absence of a dormancy phase and susceptibility to diseases and cold climatic conditions. Since these general characteristics of this GM cotton are unchanged, the inserted traits are not likely to provide a selective advantage outside of cultivation in Europe.

The EFSA GMO Panel also takes into account the fact that this application does not include cultivation of the GM cotton T304-40 within the EU so that the likelihood of cross-pollination between the imported GM cotton T304-40 and cotton crops and occasional feral cotton plants is considered to be extremely low. Even in the case that feral populations of cotton T304-40 were established or transgene flow occurred to cultivated and feral cotton, a selective advantage would occur only under infestation of sensitive pest species or application of glufosinate ammonium-based herbicides.

6.1.1.3. Interactions of the GM plant with target organisms[41]

Owing to the intended uses of cotton T304-40, which exclude cultivation, and the low level of exposure to the environment, potential interactions of the GM plant with target organisms were not considered an issue by the EFSA GMO Panel.

6.1.1.4. Interactions of the GM plant with non-target organisms[42]

Owing to the intended uses of cotton T304-40, which exclude cultivation, and as a result of the low level of exposure to the environment, potential interactions of the GM cotton with non-target organisms were not considered an issue by the EFSA GMO Panel.

However, the EFSA GMO Panel evaluated whether the Cry1Ab protein might potentially affect non-target organisms by entering the environment through manure and faeces from animals fed with this GM cotton. Owing to the specific insecticidal selectivity of Cry proteins, non-target organisms most likely to be affected by the Cry1Ab protein belong to a same or closely related taxonomic group as those of the target organisms.

Cry proteins are degraded by enzymatic activity in the gastrointestinal tract, meaning that only low amounts of Cry proteins would remain intact to pass out in faeces. This was demonstrated for Cry1Ab (Einspanier et al., 2004; Lutz et al., 2005, 2006; Wiedemann et al., 2006; Guertler et al., 2008). There would subsequently be further degradation of these Cry proteins in the manure and faeces because of intrinsic microbial proteolytic activity. In addition, there would be further degradation of Cry proteins in soil, reducing the possibility for the exposure of potentially sensitive non-target organisms. Although Cry proteins may bind to clay minerals and humic substances in soil, thereby reducing their availability to microorganisms for degradation, there are no indications of persistence and accumulation of Cry proteins from GM crops in soil (reviewed by Icoz and Stotzky, 2008). The EFSA GMO Panel is not aware of evidence of released Cry proteins from GM plants causing significant negative effects on soil micro- or macroorganisms. Considering the scope of the application, it can be concluded that the exposure of potentially sensitive non-target organisms to the Cry1Ab protein is likely to be very low and of no biological relevance.

6.1.1.5. Interaction with the abiotic environment and biogeochemical cycles[43]

Owing to the intended uses of cotton T304-40, which exclude cultivation, and 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.

[41] Technical Dossier/Sections D8 and D9.4
[42] Technical Dossier/Section D9.5
[43] Technical Dossier/Sections D9.8 and D10

6.2. Post-market environmental monitoring[44]

The objectives of a post-market environmental monitoring (PMEM) plan according to Annex VII of Directive 2001/18/EC are (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the 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 PMEM plan falls outside the mandate of EFSA. However, the EFSA GMO Panel gives its opinion on the scientific content of the monitoring plan provided by the applicant (EFSA, 2011b). The potential exposure to the environment of cotton T304-40 would be through ingestion by animals and their manure and faeces leading to exposure of gastrointestinal tract and soil microbial populations to recombinant DNA, and through accidental release into the environment of GM cotton seeds during transport and/or processing. The scope of the PMEM plan provided by the applicant is in line with the intended uses. As the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects, no case-specific monitoring is necessary.

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

The EFSA GMO Panel is of the opinion that the scope of the PMEM plan proposed by the applicant is in line with the intended uses of cotton T304-40, as the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. In addition, the EFSA GMO Panel acknowledges the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure in the case of accidental release of viable seeds of cotton T304-40. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the PMEM plan.

6.3. Conclusion

The scope of application EFSA-GMO-NL-2011-97 is for food and feed uses, import and processing and does not include cultivation. Considering the intended uses of cotton T304-40, the environmental risk assessment is concerned with indirect exposure, mainly through ingestion by animals, and their manure and faeces leading to exposure of gastrointestinal tract and soil microorganisms, and with the accidental release into the environment of viable cotton T304-40 seeds (e.g. during transport and/or processing). In the case of accidental release into the environment of viable seeds of cotton T304-40, there are no indications of an increased likelihood of spread and establishment of feral cotton T304-40 plants. Considering its intended uses as food and feed, interactions with the biotic and abiotic environment were not considered to be an issue. Risks associated with an unlikely but theoretically possible horizontal gene transfer from cotton T304-40 to bacteria have not been identified. The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of cotton T304-40 and the guidance document of the EFSA GMO Panel on PMEM of GM plants (EFSA, 2011b). In addition, the EFSA GMO Panel acknowledges the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure in cases of accidental release of viable seeds of cotton T304-40. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the PMEM plan.

[44] Technical Dossier/Section D11

CONCLUSIONS AND RECOMMENDATIONS

The molecular characterisation data establish that the GM cotton T304-40 contains a single insert consisting of copies of the *cry1Ab* and the *bar* expression cassettes. No other parts of the plasmid used for transformation are present in cotton T304-40. Bioinformatic analyses and genetic stability studies did not raise safety issues. The levels of the Cry1Ab and PAT proteins in cotton T304-40 have been sufficiently analysed.

The EFSA GMO Panel compared the composition, phenotypic and agronomic characteristics of cotton T304-40 with those of its conventional counterpart and assessed all statistically significant differences identified. The Panel came to the conclusion that no biologically relevant differences were identified in the composition or agronomic or phenotypic characteristics of cotton T304-40 as compared with its conventional counterpart, and that its composition falls within the range of estimated natural variation, except for the expression of the Cry1Ab and PAT proteins. The safety assessment of the newly expressed proteins and the whole crop included an analysis of data from analytical and bioinformatic studies, as well as *in vitro* and *in vivo* studies. Based on the information available, there is no evidence that the genetic modification might significantly change the overall allergenicity of cotton T304-40. As neither the molecular characterisation nor the compositional analysis of the GM cotton indicated unintended effects, an alteration in the toxicity properties of GM cotton seed appears to be unlikely. Nutritional equivalence of cotton T304-40 to its conventional counterparts was indicated by compositional data. The EFSA GMO Panel concludes that cotton T304-40 is as safe and nutritious as its conventional counterpart and that it is unlikely that the overall allergenicity of the whole plant is changed.

Considering the intended uses of cotton T304-40, the environmental risk assessment is concerned with indirect exposure, mainly through ingestion by animals, and their manure and faeces leading to exposure of gastrointestinal tract and soil microorganisms, and with the accidental release into the environment of viable cotton T304-40 seeds (e.g. during transport and/or processing). In the case of accidental release into the environment of viable seeds of cotton T304-40, there are no indications of an increased likelihood of spread and establishment of feral cotton T304-40 plants. Considering its intended uses as food and feed, interactions with the biotic and abiotic environment were not considered to be an issue. Risks associated with an unlikely but theoretically possible horizontal gene transfer from cotton T304-40 to bacteria have not been identified. The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of cotton T304-40 and the guidance document of the EFSA GMO Panel on PMEM of GM plants (EFSA, 2011b). In addition the EFSA GMO Panel acknowledges the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure in cases of accidental release of viable seeds of cotton T304-40. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the PMEM plan.

In conclusion, the EFSA GMO Panel considers that the information available for cotton T304-40 addresses the scientific issues indicated by the guidance document of the EFSA GMO Panel and the scientific comments raised by the Member States, and that cotton T304-40 is as safe as its comparator with respect to potential effects on human and animal health or the environment in the context of its intended uses.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of The Netherlands received on 7 April 2011 concerning a request for placing on the market of cotton T304-40 for food and feed uses, import and processing in accordance with Regulation (EC) No 1829/2003.

2. Acknowledgement letter dated 28 April 2011 from EFSA to the Competent Authority of The Netherlands.

3. Letter from EFSA to applicant dated 26 May 2011 requesting additional information under completeness check.

4. Letter from applicant to EFSA received on 7 July 2012 providing the timeline for submission of responses.

5. Letter from applicant to EFSA received on 29 August 2011 providing additional information under completeness check.

6. Letter from EFSA to applicant dated 12 September 2011 requesting additional information under completeness check.

7. Letter from applicant to EFSA received on 3 October 2011 providing additional information under completeness check.

8. Letter from EFSA to applicant dated 24 October 2011 delivering the 'Statement of Validity' for application EFSA-GMO-NL-2011-97, cotton T304-40 submitted by Bayer under Regulation (EC) No 1829/2003.

9. Letter from EFSA to applicant dated 5 December 2011 requesting additional information and stopping the clock.

10. Letter from applicant to EFSA received on 29 December 2012 providing the timeline for submission of responses.

11. Letter from applicant to EFSA received on 22 March 2012 providing additional information.

12. Letter from EFSA to applicant dated 23 April 2012 requesting additional information and maintaining the clock stopped.

13. Letter from applicant to EFSA received on 6 June 2012 providing additional information.

14. Letter from EFSA to applicant dated 9 January 2013 re-starting the clock.

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APPENDIX 1.

Table 1: Mean values for constituents in seeds of cotton T304-40 and its conventional counterpart Coker 315 harvested in the USA in 2007 (T304-40 treated or untreated with glufosinate ammonium) for which statistically significant differences were identified by the statistical analysis

Component[1]	T304-40		Conventional counterpart
	Treated	Untreated	
Ash %	3.87	3.95	3.68
Protein %	24.94	24.68	24.06
NDF %	46.41	45.31	47.81
Calcium %	0.184	0.187	0.144
Iron mg/kg	44.1	47.1	42.0
Magnesium %	0.363	0.366	0.338
Phosphorus %	0.554	0.568	0.528
Zinc mg/kg	35.6	36.3	31.7
Myristic acid % rel.	0.65	0.66	0.76
Palmitic acid % rel.	23.40	23.40	24.12
Palmitoleic acid % rel.	0.43	0.44	0.48
Stearic acid % rel.	2.76	2.71	2.48
Linoleic acid % rel.	57.14	56.96	56.18
α-Linolenic acid % rel.	0.22	NS	0.21
Arachidic acid % rel.	0.287	0.286	0.291
Lignoceric acid % rel.	< 0.12–0.12	< 0.12–0.13	< 0.12–0.24
Dihydrosterculic acid % rel.	0.138	NS	0.159
Phytic acid %	1.49	1.51	1.38

[1] Components for which statistically significant differences were identified between T304-40 (treated or untreated) and the conventional counterpart are shown. For components where statistically significant treatment × site interactions were seen, significance was considered to exist when a majority (> 50 %) of the sites showed a statistically significant difference when analysed separately. Fatty acids are in % relative to all fatty acids. NS, not statistically significant.

Table 2: Mean values for constituents in seeds of cotton T304-40 and its conventional counterpart Coker 315 harvested in the EU in 2007 (T304-40 treated or untreated with glufosinate ammonium) for which statistically significant differences were identified by the statistical analysis

Component[1]	T304-40		Conventional counterpart	Reference ranges 2007
	Treated	Untreated		
Ash %	3.96	3.91	3.81	3.28–4.28
ADF %	37.3	38.0	36.7	34.3–42.8
Calcium %	0.19	0.19	0.13	0.08–0.19
Zinc mg/kg	48.2	47.8	42.9	19.9–57
Myristic acid % rel.	0.65	0.65	0.76	0.72–1.01
Palmitic acid % rel.	22.51	22.44	23.10	20.10–24.97
Palmitoleic acid % rel.	0.45	0.45	0.51	0.50–0.74
Stearic acid % rel.	2.86	2.87	2.61	2.47–3.28
Cis-oleic acid % rel.	0.84	0.83	0.90	0.82–1.70
Oleic acid % rel.	16.94	16.98	16.67	16.96–21.12
Linoleic acid % rel.	54.12	54.16	53.77	47.26–53.62
α-Linolenic acid % rel.	0.17	0.17	0.16	0.12–0.17
Gadoleic acid % rel.	NS	0.06	0.06	0.05–0.07
Lignoceric acid % rel.	NS	0.07	0.07	0.04–0.12
Gossypol free	NS	0.55	0.57	0.45–0.83
Gossypol total	NS	1.00	1.01	0.65–1.09
Phytic acid %	1.36	1.32	1.19	0.72–1.96

[1] Components for which statistically significant differences were identified between T304-40 (treated or untreated) and the conventional counterpart are shown. For components where statistically significant treatment × site interactions were seen, significance was considered to exist when a majority (> 50 %) of the sites showed a statistically significant difference when analysed separately. Fatty acids are in % relative to all fatty acids. NS, not statistically significant.

Table 3: Mean values for constituents in seeds of cotton T304-40 and its conventional counterpart Coker 315 harvested in the EU in 2008 (T304-40 treated or untreated with glufosinate ammonium) for which statistically significant differences were identified by the statistical analysis

Component[1]	T304-40		Conventional counterpart	Reference ranges 2008
	Treated	Untreated		
Fat %	23.6	23.3	22.0	20.8–26.1
Total carbohydrate %	43.9	43.9	46.0	44.3–51.5
ADF %	40.6	NS	37.8	36.0–40.4
Calcium %	0.18	0.17	0.10	0.10–0.19
Potassium %	1.13	1.10	1.17	1.04–1.21
Zinc mg/kg	34.0	36.8	45.3	28.5–50.4
Palmitic acid % rel.	22.50	22.59	22.96	20.35–23.62
Margaric acid % rel.	0.11	0.11	0.08	0.08–0.11
Stearic acid % rel.	2.97	3.02	2.62	2.41–3.19
Oleic acid % rel.	17.43	17.32	16.60	15.83–20.79
Linoleic acid % rel.	53.89	53.87	54.90	48.63–56.14
Trans-linoleic acid % rel.	0.05	0.04	0.03	0.02–0.05
α-Linolenic acid % rel.	0.18	0.16	0.12	0.12–0.17
Gadoleic acid % rel.	0.05	0.05	0.04	0.04–0.06
Behenic acid % rel.	0.10	0.08	0.05	0.05–0.10
Lignoceric acid % rel.	0.07	0.06	0.05	0.04–0.08
Gossypol free	0.64	0.72	0.56	0.37–1.02
Gossypol total	0.82	0.89	0.71	0.49–1.30
Malvalic acid	0.39	0.38	0.31	0.23–0.55

[1] Components for which statistically significant differences were identified between T304-40 (treated or untreated) and the conventional counterpart are shown. For components where statistically significant treatment × site interactions were seen, significance was considered to exist when a majority (> 50 %) of the sites showed a statistically significant difference when analysed separately. Fatty acids are in% relative to all fatty acids. NS, not statistically significant.