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SCIENTIFIC OPINION

Scientific Opinion on application EFSA-GMO-RX-MON1445 for renewal of the authorisation for continued marketing of cottonseed oil, food additives, feed materials and feed additives produced from cotton MON 1445 that were notified as existing products under Articles 8(1)(a), 8(1)(b) and 20(1)(b) of Regulation (EC) No 1829/2003 from Monsanto[1]

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

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ABSTRACT

This scientific opinion evaluates the risk assessment for the authorisation for continued marketing of genetically modified herbicide tolerant cotton MON 1445 for food and feed produced from it. Cotton MON 1445 contains single copies of functional CP4 *epsps* and *npt*II expression cassettes and an *aad*A gene as non-functional element. Stability of the inserted DNA was confirmed over several generations. Bioinformatic analyses and levels of recombinant proteins did not reveal safety issues. No biologically relevant differences were identified in the compositional, phenotypic and agronomic characteristics of cotton MON 1445 in comparison to its conventional counterpart and its composition fell within the range of non-GM cotton varieties, except for CP4 EPSPS and NPTII proteins. No toxicity and allergenicity issues were identified regarding the newly expressed protens. Products from cotton MON 1445 do not contain viable plant parts. Insert structure in cotton MON 1445 may facilitate the stabilisation of the *npt*II gene in plasmids of environmental bacteria through double homologous recombination. However, considering the expected low frequency of gene transfer from cotton MON 1445 to bacteria compared to that between bacteria, and the very low exposure to DNA from cotton MON 1445, the EFSA GMO Panel concludes that the contribution of horizontal gene transfer to the environmental prevalence of *npt*II genes is negligible. Potential interactions of cotton MON 1445 with non-target organisms and the abiotic environment were not considered to be an issue because of low exposure levels. A post-market environmental monitoring plan is not required. The EFSA GMO Panel concludes that the information available for cotton MON 1445 addresses the questions raised by the Member States and that MON 1445-derived products are as safe as products derived from the conventional counterpart in the context of their intended uses.

[1] On request from the European Commission on application (EFSA-GMO-RX-MON1445) submitted by Monsanto, Question No EFSA-Q-2007-143, adopted on 30 November 2011.

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KEY WORDS

GMO, cotton, MON 1445, Roundup Ready®, herbicide tolerance, risk assessment, food and feed safety, environment, food and feed produced from, Regulation (EC) No 1829/2003, renewal

SUMMARY

This document provides a scientific opinion of the Scientific Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority (EFSA) on application EFSA-GMO-RX-MON1445 submitted by Monsanto under Regulation (EC) No 1829/2003[4] for renewal of the authorisation for continued marketing of (1) foods produced from cotton MON 1445 (cottonseed oil), (2) food additives produced from cotton MON 1445, and (3) feed produced from cotton MON 1445 (feed materials and feed additives).

The scope of the renewal application covers the continued marketing of cotton MON 1445:

- cottonseed oil which has been placed on the market in accordance with Article 5 of Regulation (EC) 258/97;[5]
- food additives authorised under Directive 89/107/EEC;[6]
- feed materials, and feed additives subject to Directive 70/524/EEC.[7]

After the date of entry into force of the Regulation (EC) 1829/2003, the products mentioned above were notified to the European Commission according to Articles 8(1)(a), 8(1)(b) or 20(1)(b) of this Regulation and subsequently included in the Community Register of GM food and feed.

The EFSA GMO Panel assessed cotton MON 1445 with reference to the intended uses and appropriate principles described in its Guidance Document for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006b) and its Guidance Document for renewal of authorisations of existing GMO products lawfully placed on the market (EFSA, 2006a). In delivering its Scientific Opinion, the EFSA GMO Panel considered the renewal application EFSA-GMO-RX-MON1445, additional information submitted by the applicant on request of the EFSA GMO Panel, the scientific comments submitted by Member States, and relevant scientific publications. In accordance with its Guidance Document for renewal of authorisations of existing GMO products (EFSA, 2006a), the EFSA GMO Panel has taken into account the new information, experience and data on cotton MON 1445, which became available during the authorisation period. The scientific evaluation of the risk assessment included molecular characterisation of the inserted DNA and expression of the target proteins. Evaluation of the comparative analysis of agronomic and phenotypic traits and composition was undertaken and the safety of the new proteins and the whole food/feed were evaluated with respect to potential toxicity, allergenicity and nutritional quality. An assessment of environmental impacts and the necessity of a post-market environmental monitoring plan were performed.

Cotton MON 1445 has been developed for tolerance to glyphosate-based herbicides by the introduction, via *Agrobacterium tumefaciens*-mediated (renamed as *Rhizobium radiobacter*) transformation, of a gene coding for 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4 (CP4 *epsps*). Cotton MON 1445 also carries antibiotic resistance genes coding for neomycin phosphotransferase II (NPTII) and 3′(9)-O-nucleotidyltransferase (AAD, not expressed in cotton MON 1445).

The molecular characterisation data establish that the genetically modified cotton MON 1445 contains a single T-DNA insert consisting of the *ori*V origin of replication, *npt*II expression cassette, *aad*A gene and the CP4 *epsps* expression cassette. Appropriate bioinformatic analyses of the integration site, including flanking sequences have been performed to characterise cotton MON 1445. The stability of

[4] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 1-23.
[5] Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. OJ L 43, 1-6.
[6] Council Directive 89/107/EEC of 21 December 1988 on the approximation of the laws of the Member States concerning food additives authorized for use in foodstuffs intended for human consumption. OJ L 40, 27-33.
[7] Council Directive 70/524/EEC of 23 November 1970 concerning additives in feeding-stuffs. OJ L 270, 1-17.

the genetic modification has been demonstrated over several generations. The expression of the CP4 EPSPS and NPTII proteins has been analysed sufficiently and data from various plant tissues were provided from field trials performed between 1993 and 2001 in the USA.

Based on the results of a comparative analysis of data and in the light of the field trial design and the ranges of constituent levels reported for conventional cotton varieties, the EFSA GMO Panel concludes that no biologically relevant differences were identified in the compositional characteristics of cotton MON 1445 in comparison to its conventional counterpart and that its composition fell within the range of non-GM cotton varieties, except for expressing the CP4 EPSPS and NPTII proteins. Based on the analysis of field trial data on seed and plant development, disease and pest susceptibility, reproduction and yield, the EFSA GMO Panel concludes that no biologically relevant differences were identified in the phenotypic and agronomic characteristics of cotton MON 1445 in comparison to its conventional counterpart,with the exception of the newly introduced trait. The safety of the CP4 EPSPS and NPTII proteins expressed in cotton MON 1445 is supported by bioinformatic analysis, specific studies on stability during processing, digestibility in simulated gastric and intestinal fluids and toxicity studies on mice. The potential allergenicity of the CP4 EPSPS and NPTII proteins has been assessed, and it was found unlikely that they are allergenic. The EFSA GMO Panel concludes that cotton MON 1445-derived products obtained through seed processing are as safe and nutritious as products derived from the conventional counterpart in the context of their intended use..

According to the information provided by the applicant, food and feed products produced from cotton MON 1445 have been consumed without reports of adverse effects since they have been placed on the market in the EU. Scientific publications, which became available since the previous evaluation of cotton MON 1445 by the Scientific Committee on Plants (SCP, 1998) and the UK Advisory Committee on Novel Foods and Processes (ACNFP, 2002) did not raise safety issues.

The scope of application EFSA-GMO-RX-MON1445 only covers food and feed products, produced from cotton MON 1445, which contain no viable plant parts. Therefore, there are no requirements for scientific information on environmental risks associated with the accidental release or cultivation of cotton MON 1445. No risk arising from a horizontal gene transfer of the CP4 *epsps* gene from cotton MON 1445 to bacteria has been identified. There is sequence similarity between parts of the functional insert flanking the *npt*II gene and naturally occurring bacterial plasmid sequences. Cotton MON 1445 includes two bacterial antibiotic resistance genes and other sequences of bacterial origin, which may allow double homologous recombination to plasmid sequences present in the environment. Sequence similarity suggested an increased likelihood of stabilisation of the *npt*II gene from DNA from cotton MON 1445 in bacteria. However, considering the expected low frequency of gene transfer from cotton MON 1445 to bacteria compared to that between bacteria, and the very low exposure to DNA from cotton MON 1445, the EFSA GMO Panel concludes that the contribution of horizontal gene transfer to the environmental prevalence of *npt*II genes is negligible. The assessment of horizontal gene transfer from cotton MON 1445 to bacteria does not indicate a risk to human or animal health or to the environment in the context of its intended uses. Considering the scope of the application, potential interactions of cotton MON 1445 with non-target organisms and the abiotic environment were not considered to be an issue due to the low level of exposure. A post-market environmental monitoring plan for cotton MON 1445 is not required.

In conclusion, the EFSA GMO Panel considers that information available for cotton MON 1445 addresses the questions raised by the Member States and that MON 1445-derived products are as safe as products derived from the conventional counterpart in the context of their intended uses.

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BACKGROUND

On 29 June 2007, the European Food Safety Authority (EFSA) received from the European Commission an application submitted under Regulation (EC) No 1829/2003 for renewal of the authorisation of food additives produced from cotton MON 1445 and feed produced from cotton MON 1445 (feed materials and feed additives), developed by Monsanto to provide tolerance to glyphosate-based herbicides. On 30 June 2011, the European Commission acknowledged the applicant's request to expand the scope to include renewal of authorisation of existing foods produced from cotton MON 1445 (cottonseed oil).

The scope of the renewal application, as described in the Community Register,[8] covers the continued marketing of:

- cottonseed oil notified as existing food falling within the scope of Article 8(1)(a) of Regulation (EC) 1829/2003, which is produced from a genetically modified organism (GMO) and which has been placed on the market in accordance with Article 5 of Regulation (EC) 258/97 (notification forwarded to Member States on 19 December 2002; opinion on substantial equivalence issued by the UK Advisory Committee on Novel Foods and Processes (ACNFP, 2002));

- foods additives produced from cotton MON 1445 notified as existing food additives within the meaning of Article 8(1)(b) of Regulation (EC) 1829/2003, authorised under Directive 89/107/EEC, and complying with the relevant specifications laid down under this legislation;

- existing feed produced from cotton MON 1445 (feed materials and feed additives) notified as existing feed falling within the scope of Article 20(1)(b) of Regulation (EC) 1829/2003, namely as feed materials and feed additives (subject to Directive 70/524/EEC) which are produced from a GMO.

The scope of the present renewal application covers both *Gossypium hirsutum* L. and *Gossypium barbadense* L. cotton species.

After the date of entry into force of the Regulation (EC) No 1829/2003, the products mentioned above were notified to the European Commission according to Articles 8(1)(a), 8(1)(b) or 20(1)(b) of this Regulation and subsequently included in the Community Register of GM food and feed.

Cotton MON 1445 was the subject of earlier safety assessments (ACNFP, 2002; SCP, 1998) and has been placed on the market on 1 January 1997 as food additives, feed materials and feed additives, and on 19 December 2002 as food.

After receiving the renewal application EFSA-GMO-RX-MON1445 and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed Member States as well as the European Commission and made the summary of this application publicly available on the EFSA website.[9] EFSA initiated a formal review of the renewal application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of Regulation (EC) No 1829/2003. On 29 May 2008, EFSA received additional information requested under completeness check (requested on 14 January 2008), and on 3 July 2008, 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 Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC,[10] to request their scientific opinion. The

[8] http://ec.europa.eu/food/dyna/gm_register/gm_register_auth.cfm?pr_id=5
[9] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2007-143
[10] 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.

Member State bodies had three months after the date of receipt of the valid application (until 3 October 2008) within which to make their opinion known.

The EFSA GMO Panel carried out an evaluation of the risk assessment of the renewal application of cotton MON 1445 in accordance with the appropriate principles described in its Guidance Document for the risk assessment of genetically modified (GM) plants and derived food and feed (EFSA, 2006b) and its Guidance Document for renewal of authorisations of existing GMO products lawfully placed on the market (EFSA, 2006a). In addition, the scientific comments of Member States, the additional information provided by the applicant and relevant scientific publications were taken into consideration.

The EFSA GMO Panel requested additional information from the applicant on 24 June 2008, 9 September 2008, 30 January 2009, 27 April 2009, 24 July 2009, 17 September 2009, 27 May 2010, 4 October 2010, 31 January 2011 and on 18 April 2011. The applicant provided the requested information on 14 November 2008, 23 June 2009, 3 February 2010, 3 June 2010, 8 June 2010, 14 September 2010, 2 December 2010, 11 April 2011 and on 26 July 2011.

In giving its scientific opinion on cotton MON 1445 to the European Commission, the Member States and the applicant, and in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003, EFSA has endeavoured to respect a time limit of six months from the acknowledgement of the valid application. As additional information was requested by the EFSA GMO Panel, the time-limit of six months was extended accordingly, in line with Articles 6(1), 6(2), 18(1), and 18(2) of Regulation (EC) No 1829/2003.

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

TERMS OF REFERENCE

The EFSA GMO Panel was requested to carry out a scientific assessment of cotton MON 1445 (Unique Identifier: MON-Ø1445-2) for the renewal of authorisation of (1) cottonseed oil notified as existing food falling within the scope of Article 8(1)(a) of Regulation (EC) 1829/2003, which is produced from a GMO and which has been placed on the market in accordance with Article 5 of Regulation (EC) 258/97 (notification forwarded to Member States on 19 December 2002; opinion on substantial equivalence issued by the UK Advisory Committee on Novel Foods and Processes); (2) food additives produced from cotton MON 1445 notified as existing food additives within the meaning of Article 8(1)(b) of Regulation (EC) 1829/2003, authorised under Directive 89/107/EEC, and complying with the relevant specifications laid down under this legislation; and (3) feed produced from cotton MON 1445 (feed materials and feed additives) notified as existing feed falling within the scope of Article 20(1)(b) of Regulation (EC) 1829/2003, namely as feed materials and feed additives (subject to Directive 70/524/EEC) which are produced from a GMO. 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 a scientific opinion on information required under Annex II of the Cartagena Protocol. Furthermore, the EFSA GMO Panel did not consider proposals for labelling and methods of detection (including sampling and the identification of the specific transformation event in the food/feed and/or food/feed produced from it), which are matters related to risk management.

ASSESSMENT

1. Introduction

The evaluation presented here is based on the information and risk assessment provided by the applicant in the renewal application EFSA-GMO-RX-MON1445 for continued marketing of (1) foods produced from cotton MON 1445 (cottonseed oil), (2) food additives produced from cotton MON 1445, and (3) feed produced from cotton MON 1445 (feed materials and feed additives), as well as the additional information submitted by the applicant in response to questions asked by the EFSA GMO Panel, scientific comments from Member States and relevant scientific publications. The assessment has taken into account the appropriate principles described in the EFSA GMO Guidance Document for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006b), and the EFSA GMO Guidance Document for renewal of authorisations of existing GMO products lawfully placed on the market, notified according to Articles 8 and 20 of Regulation (EC) No 1829/2003 (EFSA, 2006a).

Information in the application included (1) updated information on the comparative compositional analysis, (2) an estimation of the human and livestock exposure in Europe to cotton MON 1445, (3) an update on peer-reviewed scientific literature on cotton MON 1445, and (4) updated information on potential for allergenicity and toxicity, including updated similarity searches between the newly expressed proteins and known toxic and allergenic proteins.

The original transformed cotton MON 1445 plant was from the species *Gossypium hirsutum* L.[11] Since there are no known genetic barriers to interspecies hybridization between the tetraploid *Gossypium* species (Percival et al., 1999), the event MON 1445 could be introgressed in *Gossypium barbadense* L. through conventional breeding. On request of the EFSA GMO Panel, the applicant provided information that the composition of cottonseed from *G. barbadense* does not differ from that of *G. hirsutum* regarding nutrients, anti-nutrients and toxicants, to such an extent that a food and feed risk assessment of one of these species would not be applicable also to the other species[12]

2. Issues raised by the Member States

The comments raised by the Member States are addressed in Annex G of the EFSA overall opinion and have been considered in this scientific opinion.[13]

3. Molecular characterisation

3.1. Evaluation of relevant scientific data

3.1.1. Transformation process and vector constructs[14]

Cotton MON 1445 was developed through *Agrobacterium tumefaciens*-mediated (renamed as *Rhizobium radiobacter*) transformation of hypocotyl cells. The plasmid vector PV-GHGT07 contained two plant expression cassettes.

One of the cassettes consisted of the gene encoding the CP4 EPSPS protein from *Agrobacterium* sp. strain CP4, conferring tolerance to glyphosate-containing herbicides, under the control of the modified *Figwort mosaic virus* promoter followed by a sequence encoding the N-terminal chloroplast transit peptide from the *Arabidopsis thaliana epsps* gene. The transcription is terminated by the 3' non-

[11] Technical dossier / Section B1
[12] Additional information, April 2011
[13] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2007-143
[14] Technical dossier / Section C1

translated region of the *E9* gene. The *E9* gene encodes pea ribulose-1,5-biphosphate carboxylase small subunit.

The other cassette contained a DNA fragment derived from transposon Tn*5* consisting of the coding sequence of neomycin phosphotransferase II (*npt*II) for resistance to kanamycin and neomycin under the control of the *Cauliflower mosaic virus* (CaMV) 35S promoter and the 3' non-translated region of the nopaline synthase gene from *A. tumefaciens*. The expression of the *npt*II gene allowed selection of the transformed plant cells with kanamycin.

Furthermore, the vector PV-GHGT07 contained the origins of replication from the RK2 (*ori*V) and from pBR322 plasmids allowing replication in *A. tumefaciens* and *E. coli,* respectively. It also contained the *rop* gene, which is involved in controlling the plasmid copy number; the *bom* site for the conjugational transfer into *A. tumefaciens*; the right T-DNA border from the pTiT37 plasmid; the prokaryotic *aad*A gene from transposon Tn*7*, conferring bacterial resistance to the antibiotics spectinomycin and streptomycin and the *gox* gene encoding a synthetic glyphosate oxireductase (GOX) from *Ochrobactrum anthropi*.

3.1.2. Transgene constructs in the GM plant[15]

In cotton MON 1445, a single 6.1 kb insert is integrated that consists of the right border sequence, the CP4 *epsps* gene cassette, the *aad*A gene, the *npt*II gene cassette and part of the *ori*V. The *gox* gene, the *rop* gene and the origin of replication from pBR322 were not inserted, which was confirmed by Southern analysis.

The nucleotide sequence of the insert in cotton MON 1445 and flanking sequences extending 172 bp at the 5' end and 124 bp at the 3' end have been determined. Sequencing of the pre-insertion locus demonstrated that 67 bp of cotton genomic DNA were deleted upon insertion. Cotton MON 1445 was shown to contain one additional base pair at the 3' junction next to the T-DNA border that was not present in the parental line Coker 312.

Updated bioinformatic analyses of the 5' and 3' flanking regions did not reveal disruption of known cotton genes or the creation of novel open reading frames that would show significant similarity to known allergens or toxins.[16]

Two antibiotic resistance genes (*npt*II and *aad*A) are present in cotton MON 1445 as a consequence of the genetic modification process. The transfer of antibiotic resistance marker genes from GM plants to bacteria was therefore considered. One of the key factors determining the stabilisation rates for foreign DNA in bacteria is the presence of DNA sequence similarity, which influences the frequency of homologous recombination (EFSA, 2009). In cotton MON 1445, the *npt*II gene under the control of the CaMV 35S promoter is flanked upstream by the *aad*A gene and downstream by the broad host-range origin of replication from the RK2 plasmid (*ori*V). To analyse the possibility of homologous recombination, a bioinformatic analysis was performed with the insert against all bacterial, plasmid and viral sequences (Genbank, 2010). All hits with sufficient homology to allow homologous recombination were bacterial gene sequences with the same function as in the transgene. No cryptic targets of homologous recombination were identified. The possibility of double homologous recombination was investigated as this has been shown to be a more efficient way of stabilisation of transferred sequences than is the non-homologous illegitimate recombination (EFSA, 2009). One alignment pair was found showing in the same plasmid the presence of an *aad*A gene and *ori*V sequence with sufficient homology (*aad*A: 99 % identity in a stretch of 859 bp; *ori*V: 100 % identity in a stretch of 291 bp) to allow double homologous recombination. The hit was found in a plasmid from a bacterium isolated from activated sludge.[17] Homologous recombination involving the *aad*A gene and the *ori*V would lead to the insertion of the *npt*II gene cassette in the plasmid. This process

[15] Technical dossier / Section D2
[16] Additional information, July 2011
[17] Additional information, June 2010

would not facilitate gene transfer of the CP4 *epsps* gene, since this is outside of the DNA sequences flanked by the *aad*A and the *ori*V.

3.1.3. Information on the expression of the insert[18]

The levels of the CP4 EPSPS, NPTII, AAD and GOX proteins in cotton MON 1445 were analysed by using enzyme-linked immunosorbent assay (ELISA) from plants grown in 1993 and 1994 at six locations in the cotton growing area of the USA. In addition, trials were performed in the USA in 1998 (three lines, four locations), in 1999 (one line, four locations) and in 2001 (one line, five locations). The levels of CP4 EPSPS and NPTII proteins in seeds varied between 55 – 335 µg/g fresh weight (fw) and 0.5 – 38.1 µg/g fw, respectively (Table 1). The *aad*A gene, which is under the control of a bacterial promoter, was not expressed at protein level in any of the samples analysed. As expected, the GOX protein was not detected since the *gox* gene was shown by Southern analysis not to be present in the plant.

Table 1. Mean protein levels and ranges in cotton MON 1445 seed (µg/g fresh weight)

Year / line	CP4 EPSPS	NPTII
1993	82 58 – 117	6.7 0.5 – 10
1994	71 55 – 95	7.0 2 – 9
1998 line DP50RR	255 175 – 305	30.4 15.9 – 38.1
1998 line DP5415RR	299 235 – 335	35.1 30.1 – 37.2
1998 line DP5690RR	276 246 – 315	29.0 22.7 – 33.3
1999 line DP5415RR	138 76.9 – 281	14.8 11.3 – 20.4
2001 line SureGrow 125	110 100 – 120	16 13 – 17

3.1.4. Inheritance and stability of inserted DNA[19]

The stability of the insert across five generations was investigated by Southern analysis in plants derived from the R_0, R_3 and R_5 generations. The integrity of the functional insert was confirmed in all materials tested. The expected segregation ratio was observed over two generations, based on glyphosate susceptibility of the progeny, indicating the presence of a single Mendelian locus. The herbicide tolerance phenotype of cotton MON 1445 has been demonstrated in different genetic backgrounds under field conditions since 1993 and on a commercial scale since 1997 in the USA and other growing areas.

3.2. Conclusion

Cotton MON 1445 contains single copies of functional CP4 *epsps* and *npt*II expression cassettes and *aad*A gene as a non-functional element. Appropriate bioinformatic analyses of the integration site, including flanking sequences has been performed to characterise cotton MON 1445. The stability of the genetic modification has been demonstrated over several generations. The levels of the CP4 EPSPS and NPTII proteins have been analysed sufficiently. The event MON 1445 includes two bacterial antibiotic resistance genes and other sequences of bacterial origin, which may allow double homologous recombination of DNA released from MON 1445-derived products with plasmid sequences present in the environment (further discussed in Section 6.1.1 of this opinion).

[18] Technical dossier / Section D3
[19] Technical dossier / Section D5

4. Comparative analysis

4.1.1. Choice of comparator and production of material for the compositional assessment[20]

Seeds from cotton MON 1445 and the conventional counterpart Coker 312 were harvested from field trials performed at six locations in the major cotton growing regions of the USA in 1993 and 1994. The field trials performed in 1993 included cotton MON 1445 and Coker 312 both not treated with glyphosate. The field trial performed the following year included cotton MON 1445 plots treated with glyphosate (sprayed) as well as cotton MON 1445 and Coker 312 plots treated with other conventional herbicides (non-sprayed). Harvested cottonseeds receiving the same treatment at the six different field trial sites were pooled and processed for a comparative compositional analysis.

Additional compositional data[21] were obtained from cottonseed harvested at four field trial sites in the USA in 1999, each site having four replicates of cotton MON 1445 and its conventional counterpart. As the cotton MON 1445 event studied in these field trials had been bred into the DP5415 genetic background, the conventional counterpart in the field trials in 1999 was DP5415 instead of Coker 312. The analytical data on cottonseed from the four field trial sites in 1999 were statistically analysed both across all sites and for each field trial site individually. In addition, these field trials included 11 non-GM reference varieties to determine the range in cotton constituent levels in these field trials. The 99 % tolerance interval of this range was calculated to establish the naturally occurring range of the various cotton constituents studied. Literature values were also provided for these parameters in cottonseed (ILSI, 2007).

The harvested material and processed products were analysed for key nutrients, anti-nutrients and toxicants as defined by the OECD consensus document on compositional considerations for new varieties of cotton (OECD, 2009). Cottonseed harvested in 1993 was processed into toasted meal and refined cottonseed oil and the processed products analysed for their composition. Vitamin E (alpha-tocopherol) was reported for refined cottonseed oil produced in 1993 and crude fibre in the cottonseed harvested in 1994 and 1999.

4.1.2. Compositional analysis[22]

Statistical analysis of compositional data for cottonseed produced across all the six locations in 1993 and 1994 respectively, showed statistically significantly higher protein levels in cotton MON 1445 than in cotton Coker 312, both in glyphosate-treated and conventionally treated cotton MON 1445. The protein levels fell well within the range reported in (OECD, 2009). Furthermore, the protein level was not statistically different in MON 1445 as compared to the conventional counterpart in the material harvested in 1999. In 1993 and 1994, no differences were noted in the amino acid profiles between the cotton MON 1445 and Coker 312 lines. The amount of crude fat and ash were significantly higher in cotton MON 1445 sprayed with conventional herbicides (but not in the glyphosate-sprayed material) as compared to the conventional counterpart in 1994; no difference was noted in 1993. In cotton MON 1445 harvested in 1994 and sprayed with glyphosate a lower level of myristic acid was noted, whereas in the non-sprayed cotton MON 1445 the arachidic acid level was higher compared to levels in the Coker 312 cotton. As shown in Table 2, the level of the natural toxicant gossypol (total and free) was significantly higher in cotton MON 1445 than in the conventional counterpart Coker 312, both in field trials performed in 1993 and in 1994. The levels of total gossypol for MON 1445 and Coker 312 were within the ranges reported in (OECD, 2009) (0.5 - 1.4 % dry weight, DW) . The data on free gossypol for both MON 1445 and Coker 312 were higher than the ranges reported in (OECD, 2009) (0.5 - 0.7 % DW). The total and free gossypol levels were lower when the MON 1445 event occurred in the DP5415 genetic background (field trials in 1999)

[20] Technical dossier / Section D7.2
[21] Annex 3.4c
[22] Technical dossier / Section D7.1 and Annex 3.4c

and did not reach statistical significance in this genetic background. As no gossypol (total and free) could be detected in the refined oil and no free gossypol in the toasted meal derived from the cotton MON 1445 and Coker 312 lines (Table 2), the statistically significant differences in gossypol levels observed in the cottonseeds in the 1993 and 1994 field trials between the GM and the conventional counterpart were not considered of toxicological relevance in the context of the intended use.

Table 2. Gossypol content of cottonseed and processed products from cotton MON 1445 and the non-GM conventional counterpart obtained from field trials in the 1993, 1994 and 1999.

Year	Comparator	Total gossypol (mean % DW)			Free gossypol (mean % DW)		
		Glyphosate treated	Untreated		Glyphosate treated	Untreated	
		GM	GM	c.c.	GM	GM	c.c.
1993	*Coker 312*						
Seed						0.830*	0.695
Refined oil			ND	ND		ND	ND
Toasted meal						ND	ND
1994	*Coker 312*						
Seed		1.047*	1.023*	0.902	0.947*	0.903*	0.774
1999	*DP5415*						
Seed			0.860	0.830		0.810	0.760

c.c.: conventional counterpart
*: Statistically significant difference ($p < 0.05$)
ND: Not Detectable

In the statistical analysis of compositional data on cottonseed across sites for field trials in 1999, a statistically significant increase in the levels of palmitoleic acid, dihydrosterculic acid and iron was observed in cotton MON 1445 as compared to its conventional counterpart DP5415. In addition to these altered levels in cottonseed constituents in the over-all-sites analysis, several constituents were statistically significantly different in cotton MON 1445 and DP5415 in the per site analysis of the data. However, none of these differences were consistently found across all sites.

The EFSA GMO Panel considered the compositional data on cotton MON 1445 and its conventional counterpart in the light of the field trial design and the ranges in constituent levels reported for conventional cotton varieties. No biologically relevant differences were identified in the compositional characteristics of cotton MON 1445 in comparison to its conventional counterpart and its composition fell within the range of non-GM cotton varieties, except for CP4 EPSPS and NPTII proteins.

4.1.3. Agronomic traits and GM phenotype[23]

Agronomic and phenotypic characteristics of cotton MON 1445 and its conventional counterpart were studied in field trials conducted at four sites in the USA in 1998 and 1999, respectively. In 1998, the MON 1445 event was tested in three genetic backgrounds (DP50, DP5690 and DP5415), each having its appropriate conventional counterpart, whereas in 1999 cotton MON 1445 in the DP5415 background was tested. The agronomic and phenotypic parameters studied were related to seed and plant development, disease and pest susceptibility, reproduction and yield. In the statistical analysis across all sites in 1998, the yield of cottonseed MON 1445 was significantly higher than for the conventional counterpart DP5415.[24] This difference was not observed in the other genetic backgrounds

[23] Annex 3.4b
[24] Additional information, February 2010

and in the field trials performed in 1999. In the combined-site analysis of the data from the field trial in 1999, the stand count at day 14 was lower for cotton MON 1445 than for the conventional counterpart. No influence of stand count at day 14 was found in the field trials in 1998. In the absence of consistent differences in agronomic and phenotypic characteristics between cotton MON 1445 and its conventional counterpart, the EFSA GMO Panel concludes that no biologically relevant differences were identified in the phenotypic and agronomic characteristics of cotton MON 1445 in comparison to its conventional counterpart, with the exception of differences related to the newly introduced trait.

4.2. Conclusion

Analyses carried out on cotton MON 1445, its conventional counterpart and other non-GM cotton varieties indicate that no biologically relevant differences were identified in the compositional, phenotypic and agronomic characteristics of cotton MON 1445 in comparison to its conventional counterpart in the context of its intended uses and its composition fell within the range of non-GM cotton varieties, except for expressing the introduced trait.

5. Food/Feed safety assessment

5.1. Evaluation of relevant scientific data

5.1.1. Product description and intended uses[25]

The scope of the present application is renewal of the authorisation for continued marketing of (1) foods produced from cotton MON 1445 (cottonseed oil), (2) food additives produced from cotton MON 1445, and (3) feed produced from cotton MON 1445 (feed materials and feed additives). The possible uses of cotton MON 1445 will include the production of refined oil from seeds and cellulose from linters for use as food or food ingredients, and use of cottonseed meal, hulls and linters in animal feed.

The genetic modification of cotton MON 1445 is intended to improve agronomic performance only and is not intended to influence the nutritional properties, processing characteristics and overall use of cotton as crop.

Cotton MON 1445 was first cultivated in the USA in 1997. By 2006 cotton MON 1445 was also cultivated in Argentina, Australia, Colombia, India, Mexico, and South Africa. Cotton MON 1445, as single event or stacked with other events, is not commercially produced in any of the 27 countries of the European Community. Globally, the production of cotton MON 1445 has increased rapidly since its introduction and MON 1445-containing cotton traits reached in 2006 adoption rates exceeding 30 % of the total cotton production area in Mexico and South Africa and 80 % of the cotton cultivation area in Australia and the USA. In recent years, production of cotton MON 1445 outside Europe has levelled off or declined, being replaced by stacked cotton events containing MON 1445.

Based on import data of cottonseed oil and cottonseed meal from cotton MON 1445-producing countries into the 27 countries of the European Community over the years 2003-2005, the applicant has calculated that around 0.07 % of cottonseed oil and 0.43 % of cottonseed meal used in the EU might be derived from cotton MON 1445 and its combined trait products. It should be noted, however, that the calculations giving these figures are based on several assumptions. Because operators in the food and feed chain in some Member States of the European Community have made efforts to preferentially source non-GM products, the actual consumption of products derived from cotton MON 1445 in food and feed may vary between Member States.

[25] Annex 3.2

Based on FAO Statistics from 1997 to 2001, the human cottonseed oil consumption in Europe was calculated to be 0 and 4.9 g/person/day in central and southern Europe respectively. Assuming 0.07 % of cottonseed oil being derived from cotton MON 1445, its estimated average dietary intake by the South European consumer would be about 3.4 mg/person/day. For comparison, the average dietary intake of cottonseed oil derived from MON 1445-containing traits in the USA would be approximately 960 mg/person/day. Even if the percentage of cottonseed oil being derived from cotton MON 1445 was higher after 2005, the estimated average dietary intake would still be low in the European Union.

Animal feed is the major end use of cottonseed meal. The applicant calculated, based on data from 2005, that the maximum inclusion levels (% of the diet) of MON 1445-derived cottonseed meal in the EU would be 0, 0.022 and 0.065 % in livestock diets for pigs, broiler chickens and dairy cattle respectively. For comparison, the maximum inclusion rates of MON 1445-derived cottonseed meal in livestock diets in cotton producing countries such as the USA is up to 230-fold higher than in the European Union.

5.1.2. Effect of processing[26]

Since no biologically relevant differences were identified in the compositional, phenotypic and agronomic characteristics of cotton MON 1445 in comparison to its conventional counterpart and its composition fell within the range of non-GM cotton varieties, except for the newly expressed proteins (see Section 4.1.2), the effect of processing cottonseed MON 1445 is not expected to result in products different from those obtained after processing of conventional cotton varieties. In agreement with this assumption, no compositional differences were observed in refined cottonseed oil and toasted cottonseed meal derived from cottonseed of MON 1445 and its conventional counterpart Coker 312 harvested in the 1993 field trials, respectively.

In refined cottonseed oil, whether produced from conventional cottonseed or from seeds of cotton MON 1445, no protein or DNA was detected. The presence of newly expressed proteins in processed fractions of cotton MON 1445 is discussed in detail in Section 5.1.3.2.

Upon request of the EFSA GMO Panel to provide data on the presence/absence of DNA fragments carrying the *npt*II and the *aad*A antibiotic resistance marker (ARM) genes in the various cottonseed products intended to be placed on the market within the European Union, the applicant submitted data on the presence of genetic material in cottonseed processed products.[27] Transgenic DNA fragments spanning a functional gene length could not be detected in refined, bleached and deodorized cotton oil and in methylcellulose preparations obtained by further processing of cotton linters. It can be assumed that no ARM functional genes are present in these products. In linters and cottonseed meal, DNA fragments spanning a functional gene length are still present. In cottonseed meal their concentration is reduced to a level of about 3 % compared to cottonseed due to degradation of the DNA.

5.1.3. Toxicology[28]

5.1.3.1. Proteins used for safety assessment

The CP4 EPSPS and NPTII proteins used in safety testing were produced in *E. coli* due to the low levels of expression of these proteins in the genetically modified cotton and difficulties in purifying adequate quantities of protein from plant tissues.

The CP4 EPSPS protein expressed in *E. coli* was shown to be equivalent to that expressed in different glyphosate-tolerant cotton lines including cotton MON 1445, based on N-terminal sequencing, mobility in SDS-PAGE, Western analysis, glycosylation analysis and determination of enzymatic activity.

[26] Technical dossier / Section D7.6
[27] Additional information, December 2010
[28] Technical dossier / Section D7.8

The NPTII protein produced in *E. coli* was characterized by means of mobility in SDS-PAGE, Western analysis, amino acid sequencing, protein glycosylation and enzymatic activity. The NPTII protein expressed in *E. coli* was found to be equivalent to the NPTII protein expressed in cotton MON 1445 based on N-terminal sequencing.

E. coli produced CP4 EPSPS and NPTII proteins were shown to be structurally and functionally equivalent to the plant produced proteins. Based on the identified similarity in structure and function, the EFSA GMO Panel accepts the use of the CP4 EPSPS and NPTII proteins expressed in *E. coli* for the safety testing of the CP4 EPSPS and NPTII proteins present in cotton MON 1445.

5.1.3.2. Toxicological assessment of expressed novel proteins in cotton MON 1445

CP4 EPSPS

Since EPSPS enzymes occur in plants, fungi, and microorganisms, humans have a long history of dietary exposure to these proteins. No adverse effects have been reported with their intake. Previous applications for placing on the market glyphosate tolerant crops have included safety assessments of the CP4 EPSPS protein and no safety concerns have been identified. The EFSA GMO Panel is of the opinion that no new scientific data have emerged which call for a change of this scientific opinion.

(a) Acute toxicity testing

No adverse effects were observed in an acute oral toxicity study using CD-1 mice administered the CP4 EPSPS protein produced in *E. coli* by oral gavage at dosages up to 572 mg/kg body weight (bw).

(b) Degradation in simulated digestive fluids

The stability of the CP4 EPSPS protein purified from *E. coli* was studied in simulated gastric (SGF, pH 1.2) and intestinal fluids (SIF, pH 7.5). The data demonstrated that CP4 EPSPS was degraded to half of the original content in less than 15 sec in SGF and less than 10 min in SIF, as confirmed by Western analysis. Enzymatic activity of CP4 EPSPS was not detected in SGF after 2 min, and less than 9 % of the activity remained after 285 min in SIF.

(c) Effect of processing

The level of active CP4 EPSPS was evaluated in processed cottonseed meals prepared from cotton MON 1445. CP4 EPSPS enzymatic activity could be measured in seed and full fat flour, but was lost in toasted meal. Only traces of CP4 EPSPS could be detected in toasted meal by Western analysis. Western analysis also revealed that the CP4 EPSPS protein could be detected in combed lint (mechanically cleaned raw lint) at about 0.5 µg/g of protein, but not at all in processed linter (LOD = 0.1 µg/g of protein).

(d) Bioinformatic studies

Bioinformatics-supported comparison of the amino acid sequence of the CP4 EPSPS protein expressed in cotton MON 1445 with amino acid sequences contained in protein databases were performed in 2009.[29] No relevant similarities between the sequence of the CP4 EPSPS protein and sequences of toxic proteins were found.

NPTII

The NPTII protein has been the subject of previous safety assessments in connection with the evaluation of applications of other NPTII-expressing genetically modified crops, including the maize events MON 863, MON 863 x MON 810 and MON 863 x MON 810 x NK603 and the potato EH92-

[29] Additional information, February 2010

527-1. In none of these cases safety concerns were identified. The issue of potential horizontal gene transfer was recently addressed in a scientific opinion by the EFSA GMO Panel (EFSA, 2009) and assessed in more details in Section 6.1.1 of this scientific opinion.

(a) Acute toxicity testing

In an acute oral toxicity study, the NPTII protein expressed in *E. coli* did not induce adverse effects in mice after administration by gavage at dosages up to 5000 mg/kg bw.

(b) Degradation in simulated digestive fluids

The *E.coli*-produced NPTII protein is readily degraded in gastric and intestinal fluids, as demonstrated by Western analysis of SDS-PAGE gels. In 10 sec, the NPTII protein is degraded in simulated gastric (SGF, pH 1.2) and in 5 min in simulated intestinal fluids (SIF, pH 7.5). Using an enzymatic activity assay, the NPTII protein lost activity completely within 2 min incubation in SGF, the shortest incubation time used in the assay, and after 15 min incubation in SIF.

(c) Effect of processing

The level of active NPTII was evaluated in processed cottonseed meals prepared from cotton MON 1445. Traces of NPTII were found in full fat flour but not detected anymore after further processing of the meal as demonstrated by ELISA, Western blotting and enzymatic assay.

(d) Bioinformatic studies

The amino acid sequence similarity of the NPTII protein to amino acid sequences of proteins in publicly available databases were evaluated using bioinformatics tools in 2009.[30] No relevant similarities between the sequence of the NPTII protein and sequences of toxic proteins were found.

5.1.3.3. Toxicological assessment of new constituents other than proteins

No new constituents other than CP4 EPSPS and NPTII proteins are expressed in cotton MON 1445 and no relevant changes in the composition of cotton MON 1445 were detected in the comparative compositional analysis (see Section 4.1.2).

5.1.3.4. Toxicological assessment of the whole GM food/feed

The comparative analysis concluded that no biologically relevant differences were identified in the compositional, phenotypic and agronomic characteristics of cotton MON 1445 in comparison to its conventional counterpart and that its composition fell within the range of non-GM cotton varieties, except for expressing the introduced trait. Also the molecular characterisation provided no indications of unintended effects of the genetic modification. According to the EFSA GMO Panel guidance document, no animal safety studies with the whole food/feed are required under these conditions (EFSA, 2006b).

5.1.4. Allergenicity[31]

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 (Codex Alimentarius, 2009; EFSA, 2006a, 2010).

[30] Additional information, February 2010
[31] Technical dossier / Section D7.9

5.1.4.1. Assessment of allergenicity of the newly expressed proteins

Cottonseed oil and processed cotton linters are the primary cotton products used for human food. Analysis of cotton products derived from cotton MON 1445 confirmed that there is no detectable level of protein in both cottonseed oil and processed cotton linters: no significant human consumption of these proteins from cotton foods and food ingredients is then expected.

A bioinformatics-supported comparison of the amino acid sequence of the CP4 EPSPS and NPTII proteins with the sequences of known allergens, gliadins and glutenins collected in an updated proprietary database based on the FARRP database, has been performed.[32] This analysis included both overall sequence alignments using the FASTA algorithm and searches for short identical stretches of at least eight contiguous amino acids. In the overall sequence alignment no identity higher than 35 % in polypeptides of 80 or more amino acids was found between the CP4 EPSPS or the NPTII protein and known allergens. Similarly, no identical sequence of eight contiguous amino acids was detected. These results indicate the lack of both structurally and immunologically relevant similarities between the CP4 EPSPS and the NPTII protein and known allergens.

Furthermore, the potential allergenicity of the CP4 EPSPS and NPTII proteins have been assessed previously and it was found unlikely that they are allergenic, among others based on their fast degradation and the absence of any significant similarity with known protein allergens. The EFSA GMO Panel considers that the newly expressed proteins are unlikely to be allergenic under the intended conditions of exposure.

5.1.4.2. 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 newly introduced genes in the genome of the recipient, for example through qualitative or quantitative modifications of the pattern of expression of endogenous proteins. This issue does not appear relevant to the EFSA GMO Panel since cotton is not considered to be a common allergenic food, and only rare cases of occupational allergy have been reported (Atkins, 1988; Malanin, 1988). Furthermore, the main cottonseed product in human food, cottonseed oil, is highly purified and contains negligible levels of proteins, if any. In general, edible oils that are refined, bleached and deodorized do not appear to pose a risk to allergic individuals, as they contain virtually no proteins. Also in cellulose from cottonseed linters for use as food or food ingredients the protein level is negligible.

In the context of the present application the EFSA GMO Panel considers it unlikely that any interactions between the newly expressed proteins and metabolic pathways of cotton would alter the pattern of expression of endogenous proteins and thereby significantly change the overall allergenicity of the whole plant.

5.1.5. Nutritional assessment of GM food/feed[33]

A feeding study was performed on 200 catfish fed for 10 weeks a diet containing 20 % w/w processed meal obtained from either cotton MON 1445 or its conventional counterpart Coker 312.[34] In the study no statistically significant differences were observed in the fish fed cottonseed meal containing cotton MON 1445 and Coker 312, respectively, regarding weight gain, feed intake, feed conversion ratios, and survival. Body composition data were in the normal range apart from slightly lower fat and slightly higher protein levels in fish fed cottonseed meal from cotton MON 1445. These minor differences were not considered biologically relevant. The EFSA GMO Panel concludes that the data provided support the view that cottonseed meal derived from cotton MON 1445 is as nutritious as that derived from its conventional counterpart.

[32] Additional information, February 2010.
[33] Technical dossier / Section D7.10
[34] Annex 3.5e

5.1.6. Post-market monitoring of GM food/feed

The outcome of the risk assessment indicates that cotton MON 1445-derived products obtained through processing are as safe as those derived from its conventional counterpart. In addition, cottonseed meal derived from cotton MON 1445 was found to be as nutritious as that derived from its conventional counterpart. Therefore, and in line with the Guidance document (EFSA, 2006b), the EFSA GMO Panel is of the opinion that post-market monitoring of the GM food/feed is not necessary.

5.2. Conclusion

The safety of the CP4 EPSPS and NPTII proteins is supported by bioinformatics analysis and investigations on stability, digestibility and toxicity. The potential allergenicity of the CP4 EPSPS and NPTII proteins has been assessed, and it was found unlikely that they are allergenic. As neither the molecular characterisation nor the compositional analysis of the GM-cotton indicated any unintended effects, an alteration in allergenic properties of the GM-cottonseed appears to be unlikely. A feeding study on catfish confirmed that cottonseed meal of cotton MON 1445 is as nutritious as meal of its conventional counterpart.

The EFSA GMO Panel concludes that cotton MON 1445-derived products obtained through processing are as safe and nutritious as products derived from the conventional counterpart in the context of their intended use.

6. Environmental risk assessment and post-market environmental monitoring plan

6.1. Environmental risk assessment

The scope of application EFSA-GMO-RX-MON1445 is for food and feed products produced from cotton MON 1445; the scope of this application only includes products produced from cotton MON 1445 which contain no viable plant parts. Considering the intended uses of cotton MON 1445, the environmental risk assessment is concerned with the indirect exposure through manure and faeces from animals fed with cotton products from cotton MON 1445. There are no requirements for scientific information on environmental safety assessment of accidental release or cultivation of cotton MON 1445.

6.1.1. Potential for gene transfer[35]

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer of DNA, or vertical gene flow via seed dispersal and cross-pollination. Considering the intended uses of cotton MON 1445, there are no requirements for scientific information on environmental safety assessment of accidental release or cultivation of cotton MON 1445. Therefore, vertical gene flow via seed dispersal and cross-pollination is not considered.

6.1.1.1. Plant to bacteria gene transfer

The recombinant DNA insert in cotton MON 1445 could hypothetically be acquired through horizontal gene transfer (HGT) by bacteria. However, 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 bacteria) does not occur at quantifiable levels (EFSA, 2009). The hypothetical HGT of recombinant plant DNA to bacteria requires a genetic recombination mechanism, which, in theory, might be homologous or illegitimate recombination. The exposure of bacteria to the recombinant DNA fraction of plants should also be assessed in the context of their continuously ongoing exposure to a wide variety of other naturally occurring sources of DNA.

[35] Technical dossier / Section D9.2

The probability and frequency of HGT of plant DNA (including the recombinant DNA fraction) to exposed bacteria in the environment is determined by the following factors: (1) the amount and quality of plant DNA accessible to bacteria in relevant environments; (2) the presence of bacteria with a capacity to develop genetic competence for transformation (to take up extracellular DNA); (3) the mechanism of genetic recombination by which the plant DNA can be incorporated and thus stabilized in the bacterial genome (including chromosomes or plasmids); and (4) the mobility of the plant DNA in bacterial recipients (i.e. whether they are located on chromosomes or mobile genetic elements such as plasmids).

Furthermore, the risk assessment of an impact of rare HGT events considers the potential expression of the recombinant plant DNA in the bacterial cells, and most importantly, the selective advantage conferred by acquisition of recombinant DNA. Finally, the source of the recombinant DNA inserted into the GM plant is considered because many plant transgenes have been derived from the genomes of various soil bacteria. Information on the prevalence of similar genes and their encoded phenotypes within natural microbial communities is taken into account to understand alternative and naturally occurring exposure sources to the same genetic traits.

Hazard identification and characterisation

The CP4 *epsps* gene originates from the soil bacterium *Agrobacterium tumefaciens*. In cotton MON 1445, it is under the control of a plant virus promoter. The activity of plant virus promoters in unrelated organisms such as bacteria cannot be excluded, but in the unlikely event that the CP4 *epsps* gene and its regulatory elements are taken up by bacteria, no selective advantage is anticipated, because EPSPS encoding genes are already occurring in various bacterial species in the environment. The expected wide environmental presence of genetically diverse natural variants of EPSPS encoding genes, the use of regulatory sequences optimised for expression in eukaryotes, and the absence of any selective advantage, except in the presence of glyphosate-based herbicides, suggest it is highly unlikely that the CP4 *epsps* transfers and establishes in the genome of bacteria in the environment or human and animal digestive tracts (EFSA, 2009).

As described in Section 3.1.2., bioinformatic analysis indicates the possibility of double homologous recombination between the *aad*A gene and the *ori*V present in cotton MON 1445 with the same sequences present in bacterial plasmids isolated from activated sludge. This homologous recombination would lead to the replacement of the genes in such plasmids between the two recombination sites by the *npt*II gene cassette as present in the DNA of cotton MON 1445 and thus, the acquisition of novel genetic information. The stabilisation rate of the *npt*II gene cassette in such bacteria is estimated from laboratory experiments with comparable constructs to be increased about 10^9-10^{10} times compared to stabilisation by the process of illegitimate recombination encountered for constructs where no flanking homology to bacterial sequences has been introduced (De Vries and Wackernagel, 2002; Hülter and Wackernagel, 2008). In addition to the double homologous recombination involving flanking regions of transgenes, homologous recombination may theoretically also occur between single transgenes and their natural counterparts in bacteria, i.e., the *aad*A, *npt*II and CP4 *epsps*. Such substitutive recombination, however, would not lead to the acquisition of an additional trait, since only nucleotide substitutions with existing genes would be expected. The potential for such replacements should be considered in the context of naturally occurring homologous recombination in bacteria. Furthermore, illegitimate recombination events would also be theoretically possible, but they have not been detected even in laboratory studies in which bacteria have been exposed to high concentrations of DNA from GM plants (reviewed by EFSA, 2009) and are therefore not considered to significantly contribute to the HGT process.

Expression of the *npt*II gene under the control of CaMV 35S promoter has been demonstrated in bacteria (Assaad and Signer, 1990; Lewin et al., 1998). Therefore, oral treatment with kanamycin or neomycin may create a selective advantage for the transformed bacterial cells with the capability to express the *npt*II-encoded neomycin phosphotransferase II and could enhance further spread of *npt*II between bacteria by transformation or conjugation. The indicated uses of kanamycin or neomycin or

similar substances include gut irrigation and the treatment of encephalopathy in humans (neomycin) and treatment of diarrhoea in farm animals and aerosol administration for respiratory infections in humans and animals (EFSA, 2009).

The hazard identification and characterisation indicates that HGT of the *npt*II gene cassette of cotton MON 1445 could lead to kanamycin and neomycin resistant bacteria emerging in some environments, especially in the gastrointestinal tract or faeces, under selective conditions (usage of the corresponding antibiotic).

Exposure characterisation

DNA is a common component of many food and feed products derived from plants. During processing, the DNA of the plant material for food and feed may substantially be degraded or removed. Considering the scope of this application (see Terms of reference), products that are covered in this application include oil for food and feed; meals, cake and hulls for feed; and linters and derived products (e.g. viscose, food casings, cellulose esters and ethers) for food. Based on the information provided by the applicant and knowledge from scientific literature it can be expected that recombinant DNA is still present in cottonseed meal and linters. However, DNA was not detected in methylcellulose or oil.[36] Experimental evidence was provided that processing reduced the content of transgenic DNA spanning the *npt*II gene cassette in the cottonseed meal to a level of 1.6 to 5.1 % of what is present in unprocessed cottonseed.[37]

In case of products containing DNA, the main route of exposure to potential bacterial recipients is in the gastrointestinal systems of humans or animals. DNA present in food and feed is substantially degraded through digestion in the human and animal gastrointestinal tracts (Rizzi et al., 2012). The highest exposure is expected for unprocessed linters, because it is expected to contain the highest amount of transgenic DNA. Exposure is also possible for products in which the transgenic DNA is much degraded but the full gene length transgenic DNA could still be present at a lower level, such as for cottonseed meal. No exposure is expected from highly processed and refined products, such as cottonseed oil and methylcellulose, which cover all products of cotton MON 1445 relevant for human consumption. In animal feeding, cotton products are only used in small amounts in the EU (FEDIOL, online), mainly due the presence of gossypol, which is highly toxic for non-ruminants (Verstraete, 2011).[38] Because of low dietary amounts and degradation in the gastrointestinal tract and faeces, manure of animals fed with cotton MON 1445 will only contain negligible amounts of transferable recombinant DNA. Bacteria in soil or surface waters could be exposed to DNA from cotton MON 1445 by manure. Compared to usage as defined in the scope of this application, such exposure will be highly limited.

The probability of gene transfer depends on the presence of bacteria with a capacity to develop genetic competence for transformation, *i.e.*, to take up and recombine extracellular DNA. Several bacterial species with the potential to develop competence belong to the common gut microbial community (EFSA, 2009; Rizzi et al., 2012). However, actual competence development and transformation of such bacteria by genomic DNA of plants has not yet been observed in the lower gastrointestinal tract even with optimized model systems providing a selective advantage (EFSA, 2009; Nordgård et al., 2007; Rizzi et al., 2012). In contrast, some studies have shown that introduced bacteria can be naturally transformed in the oral cavity of humans and animals (Duggan et al., 2000, 2003; Mercer et al., 1999a, 1999b, 2001).

[36] Additional information, December 2010
[37] Additional information, December 2010
[38] Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. OJ L 140, 10-22

Risk characterisation

Gastrointestinal bacteria of humans and animals, in particular ruminants, are expected to be exposed to the *aad*A–*npt*II–oriV DNA fragment from cotton MON 1445 by the consumption of linters (consumed by humans and animals) and cottonseed meal (consumed by animals). Cottonseed meal contains mainly fragmented DNA with a size smaller than that of the above mentioned fragments.[39] In addition, DNA is further degraded in the gastrointestinal tract of animals (Jonas et al., 2001; Van den Eede et al., 2004). Furthermore, these products are only fed to animals in low amounts in the EU (FEDIOL, online; Verstraete 2011).

The *aad*A and *ori*V sequences that flank the *npt*II gene in cotton MON 1445 are present in naturally-occurring bacteria in an arrangement which would allow double homologous recombination. The theoretical probability of horizontal transfer of the transgene sequences into bacteria is therefore higher compared to plant transgenes that do not have such flanking DNA sequences. The genetic composition of the inserted DNA in cotton MON 1445 would thereby lead to HGT to bacteria harbouring *aad*A and *ori*V sites in their DNA. Since such recombination sites can be located on mobile genetic elements, rare transfer of *npt*II from plant material to bacteria could theoretically be followed by higher frequency conjugative gene transfer to other bacteria and, thus, contribute to establishment of the *npt*II-encoded resistance trait in environmental bacterial populations.

The contribution of HGT of the recombinant *npt*II gene to the development and proliferation of antibiotic resistant bacteria should be seen in the context of the naturally ongoing resistance gene transfer between bacteria, which is several orders of magnitude more frequent (Brigulla and Wackernagel, 2010). The contribution of the frequency of HGT of the recombinant *npt*II gene must likewise be regarded relative to the natural distribution and prevalence of *npt*II genes on mobile genetic elements in bacteria. Bacteria carrying *npt*II on mobile genetic elements are found in various environments, although with large spatial and temporal fluctuations (EFSA, 2009). Moreover, other resistance genes than *npt*II also lead to the distribution and prevalence of kanamycin and neomycin resistant bacteria in various environments.

There is limited information about the spatial and temporal variability in the selective conditions which would favour antibiotic-resistant bacteria, and in the occurrence, transferability and distribution of *npt*II genes in different environments. Also, there is a lack of experimental data on HGT from cotton MON 1445.

Conclusion

The environmental risk assessment indicates no risk arising from a HGT of the *aad*A and CP4 *epsps* gene from cotton MON 1445 to bacteria because of a highly limited potential for transfer. However, it reveals that for products from cotton MON 1445 containing transgenic DNA, there is an increased likelihood of stabilisation of the *npt*II gene from plant DNA in bacteria compared to plants not including sites for double homologous recombination. This increased likelihood of transfer must, however. be seen in the context of the gene transfer efficiencies between bacteria, which remains several orders of magnitude higher.

Low level exposure is expected for bacteria present in the gastrointestinal tracts of humans and animals. Considering the low level of exposure and the expected low frequency of gene transfer from MON 1445 to bacteria compared to that between bacteria, the GMO Panel concludes that the contribution of HGT to the environmental prevalence of *npt*II genes is negligible. In summary, the analysis of HGT from cotton MON 1445 to bacteria does not indicate a risk to human or animal health or to the environment in the context of its intended uses.

[39] Additional information, December 2010

6.1.2. Interactions of the GM plant with target organisms[40]

Interactions of cotton MON 1445 with target organisms were not considered an issue by the EFSA GMO Panel as there are no target organisms.

6.1.3. Interactions of the GM plant with non-target organisms[41]

Due to the intended uses of cotton MON 1445, which exclude cultivation and import of viable plant parts, interactions of cotton MON 1445 with non-target organisms were not considered an issue by the EFSA GMO Panel.

6.1.4. Interactions of the GM plant with the abiotic environment[42]

Due to the intended uses of cotton MON 1445, which exclude cultivation and import of viable plant parts, interactions of cotton MON 1445 with the abiotic environment were not considered an issue by the EFSA GMO Panel.

6.1.5. Post-market environmental monitoring[43]

Considering the scope of the application EFSA-GMO-RX-MON1445 for food and feed materials produced from cotton MON 1445, a post-market environmental monitoring plan for cotton MON 1445 is not required.

6.2. Conclusion

Cotton MON 1445 is being assessed for food and feed produced from cotton MON 1445; the scope only includes products produced from cotton MON 1445 which contain no viable plant parts. Therefore, there are no requirements for scientific information on environmental risks associated with the accidental release or cultivation of cotton MON 1445. No risk arising from a horizontal gene transfer of the CP4 *epsps* gene from cotton MON 1445 to bacteria has been identified. A hazard of an increased likelihood of stabilisation of the *npt*II gene from cotton MON 1445 in bacteria was postulated. However, considering the expected low frequency of gene transfer from cotton MON 1445 to bacteria compared to that between bacteria, and the very low exposure to DNA from cotton MON 1445, the EFSA GMO Panel concludes that the contribution of horizontal gene transfer to the environmental prevalence of *npt*II genes is negligible. The analysis of horizontal gene transfer from cotton MON 1445 to bacteria does not indicate a risk to human or animal health or to the environment in the context of its intended uses. Considering the scope of the application, potential interactions of cotton MON 1445 with non-target organisms and the abiotic environment were not considered to be an issue due to the low level of exposure. A post-market environmental monitoring plan for cotton MON 1445 is not required.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The EFSA GMO Panel was requested to deliver a scientific opinion for renewal of the authorisation for continued marketing of existing products from cotton MON 1445 (references EFSA-GMO-RX-MON1445) under Regulation (EC) No 1829/2003. The scope of this application covers the continued marketing of (1) foods produced from cotton MON 1445 (cottonseed oil), (2) food additives produced from cotton MON 1445, and (3) feed produced from cotton MON 1445 (feed materials and feed additives) which were lawfully placed on the market in the European Community before the date of entry into force of Regulation (EC) No 1829/2003 and included in the Community Register of GM food and feed.

[40] Technical dossier / Section D8
[41] Technical dossier / Section D9
[42] Technical dossier / Section D10
[43] Technical dossier / Section D11

In delivering its scientific opinion, the EFSA GMO Panel considered the renewal application EFSA-GMO-RX-MON1445, additional information submitted by the applicant on request of the EFSA GMO Panel, the scientific comments submitted by Member States, and relevant scientific publications. In accordance with its Guidance Document for renewal of authorisations of existing GMO products (EFSA, 2006a), the EFSA GMO Panel has taken into account the new information, experience and data on cotton MON 1445, which became available during the authorisation period.

The EFSA GMO Panel is of the opinion that the molecular characterisation data provided for cotton MON 1445 are sufficient to conclude on this part of the risk assessment. Cotton MON 1445 contains single copies of functional CP4 *epsps* and *npt*II expression cassettes and *aad*A gene as non-functional element. The bioinformatic analyses of the inserted DNA and the flanking regions do not raise safety concerns. The levels of CP4 EPSPS and NPTII proteins in cotton MON 1445 have been analysed and the stability of the genetic modification and the corresponding phenotype have been demonstrated.

In the comparative analysis no biologically relevant differences were identified in the compositional, phenotypic and agronomic characteristics of cotton MON 1445 in comparison to its conventional counterpart and that its composition fell within the range of non-GM cotton varieties, except for the presence of newly expressed CP4 EPSPS and NPTII proteins. The safety of the CP4 EPSPS and NPTII proteins is supported by bioinformatics analysis and investigations on stability, digestibility, toxicity and allergenicity. The EFSA GMO Panel considers that MON 1445-derived products are as safe and nutritious as products derived from the conventional counterpart in the context of their intended use.

The scope of application EFSA-GMO-RX-MON1445 only covers food and feed products derived from cotton MON 1445, which contain no viable plant parts. Therefore, there are no requirements for scientific information on environmental risks associated with the accidental release or cultivation of cotton MON 1445. No risk arising from a HGT of the CP4 *epsps* gene from cotton MON 1445 to bacteria has been identified. There is sequence similarity between parts of the functional insert flanking the *npt*II gene and naturally occurring bacterial plasmid sequences. Cotton MON 1445 includes two bacterial antibiotic resistance genes and other sequences of bacterial origin, which may allow double homologous recombination to plasmid sequences present in the environment. Sequence similarity suggested an increased likelihood of stabilisation of the *npt*II gene from DNA from cotton MON 1445 in bacteria. However, considering the expected low frequency of gene transfer from cotton MON 1445 to bacteria compared to that between bacteria, and the very low exposure to DNA from cotton MON 1445, the EFSA GMO Panel concludes that the contribution of HGT to the environmental prevalence of *npt*II genes is negligible. The assessment of HGT from cotton MON 1445 to bacteria does not indicate a risk to human or animal health or to the environment in the context of its intended uses. Considering the scope of the application, potential interactions of cotton MON 1445 with non-target organisms and the abiotic environment were not considered an issue due to the low level of exposure. A post-market environmental monitoring plan for cotton MON 1445 is not required.

In conclusion, the EFSA GMO Panel considers that information available for cotton MON 1445 addresses the questions raised by the Member States and that cotton MON 1445-derived products are as safe as products derived from the conventional counterpart in the context of their intended uses.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the European Commission, received 29 June 2007, concerning a request to renew the authorisation for placing on the market of cotton MON 1445 in accordance with Articles 8(1)(b), 20(1)(b) of Regulation (EC) No 1829/2003 submitted by Monsanto.

2. Acknowledgement letter, dated 20 July 2007, from EFSA to the European Commission.

3. Letter from EFSA to applicant, dated 14 January 2008, requesting additional information under completeness check.

4. Letter from applicant to EFSA, received 19 February 2008, providing the timeline for submission of responses.

5. Letter from applicant to EFSA, received 30 April 2008, changing the timeline for submission of responses.

6. Letter from applicant to EFSA, received 29 May 2008, providing additional information under completeness check.

7. E-mail from EFSA to applicant, dated 25 June 2008, requesting additional information under completeness check.

8. Letter from applicant to EFSA, received 2 July 2008, providing additional information under completeness check.

9. Letter from EFSA to applicant, dated 3 July 2008, delivering the 'Statement of Validity' for application EFSA-GMO-RX-MON1445, cotton MON 1445 submitted under Regulation (EC) No 1829/2003 by Monsanto.

10. Letter from EFSA to applicant, dated 9 September 2008, requesting additional information and stopping the clock.

11. Letter from applicant to EFSA, received 21 October 2008, providing the timeline for submission of responses.

12. Letter from applicant to EFSA, received 14 November 2008, providing additional information.

13. Letter from EFSA to applicant, dated 30 January 2009, requesting additional information and maintaining the clock stopped.

14. Letter from applicant to EFSA, received 12 March 2009, providing the timeline for submission of responses.

15. Letter from EFSA to applicant, dated 27 April 2009, requesting additional information and maintaining the clock stopped.

16. Letter from applicant to EFSA, received 8 June 2009, providing the timeline for submission of responses.

17. Letter from applicant to EFSA, received 23 June 2009, providing additional information.

18. Letter from applicant to EFSA, received 6 July 2009, changing the timeline for submission of responses.

19. Letter from EFSA to applicant, dated 24 July 2009, requesting additional information and maintaining the clock stopped.

20. Letter from applicant to EFSA, received 7 September 2009, providing the timeline for submission of responses.

21. Letter from EFSA to applicant, dated 17 September 2009, requesting additional information and maintaining the clock stopped.

22. Letter from applicant to EFSA, received 13 October 2009, changing the timeline for submission of responses.

23. Letter from applicant to EFSA, received 30 October 2009, providing the timeline for submission of responses.

24. Letter from applicant to EFSA, received 3 February 2010, providing additional information.

25. Letter from EFSA to applicant, dated 27 May 2010, requesting additional information and maintaining the clock stopped.

26. Letter from applicant to EFSA, received 3 June 2010, providing additional information.

27. Letter from applicant to EFSA, received 8 June 2010, providing additional information.

28. Letter from applicant to EFSA, received 15 July 2009, providing the timeline for submission of responses.

29. Letter from EFSA to applicant, dated 2 August 2010, requesting clarifications concerning additional information provided.

30. Letter from applicant to EFSA, received 14 September 2010, providing additional information.

31. Letter from applicant to EFSA, received 15 September 2010, providing clarifications.

32. Letter from EFSA to applicant, dated 4 October 2010, requesting additional information and maintaining the clock stopped.

33. Letter from applicant to EFSA, received 24 November 2010, providing the timeline for submission of responses.

34. Letter from applicant to EFSA, received 2 December 2010, providing additional information.

35. Letter from EFSA to applicant, dated 31 January 2011, requesting additional information and maintaining the clock stopped.

36. Letter from applicant to EFSA, received 21 March 2011, providing the timeline for submission of responses.

37. Letter from applicant to EFSA, received 11 April 2011, providing additional information.

38. Letter from EFSA to applicant, dated 18 April 2011, requesting additional information and maintaining the clock stopped.

39. Letter from applicant to EFSA, received 6 June 2011, providing the timeline for submission of responses.

40. Letter from applicant to EC, dated 16 June 2011, requesting an expansion of the scope of the application EFSA-GMO-RX-MON1445.

41. Letter from EC to applicant, dated 30 June 2011 acknowledging the receipt of the letter requesting an expansion of the scope of the application EFSA-GMO-RX-MON1445.

42. Letter from applicant to EFSA, received 26 July 2011, providing additional information.

43. Letter from EC to EFSA, received 1 August 2011, providing the updated summary of the application EFSA-GMO-RX-MON1445.

44. Letter from EFSA to applicant, dated 16 September 2011, restarting the clock.

45. Letter from EFSA to applicant, dated 21 September 2011, requesting additional information and stopping the clock.

46. Letter from applicant to EFSA, received 23 September 2011, providing additional information.

47. Letter from EFSA to applicant, dated 28 September 2011, restarting the clock.

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