SCIENTIFIC OPINION

Scientific Opinion on applications EFSA-GMO-UK-2005-09 and EFSA-GMO-RX-MON531×MON1445 for the placing on the market of food and feed produced from or containing ingredients produced from insect-resistant and herbicide-tolerant genetically modified cotton MON 531 × MON 1445,[1] and for the renewal of authorisation of existing products produced from cotton MON 531 × MON 1445,[2] both under Regulation (EC) No 1829/2003 from Monsanto

EFSA Panel on Genetically Modified Organisms (GMO)[3,4]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

This scientific opinion evaluates the risk assessment for the authorisation for (continued) marketing of genetically modified insect-resistant and herbicide-tolerant cotton MON 531 × MON 1445 for food and feed produced from it. Cotton MON 531 × MON 1445 was produced by conventional crossing methods, and the stack is homozygous for the newly introduced traits. The integrity and the stable co-inheritance of the parental events were demonstrated in the stack. Molecular characterisation did not reveal safety issues. No biologically relevant differences were identified in the compositional, phenotypic and agronomic characteristics of cotton MON 531 × MON 1445 in comparison with its conventional counterpart and its composition fell within the range of non-GM cotton varieties, except for the Cry1Ac, CP4 EPSPS and NPTII proteins. No safety issues were identified with regard to toxicity and allergenicity of food and feed produced from cotton MON 531 × MON 1445. A feeding study in catfish confirmed that toasted cottonseed meal from MON 531 × MON 1445 is as nutritious as toasted cottonseed meal from its conventional counterpart and four commercial non-GM cotton varieties. Products from cotton MON 531 × MON 1445 do not contain viable plant parts. The insert structure in cotton MON 531 × MON 1445 may facilitate the stabilisation of the *npt*II gene in plasmids of environmental bacteria through double homologous recombination. However, considering the overall limited occurrence of horizontal transfer of DNA in plant material to bacteria, and the very low exposure to DNA from cotton MON 531 × MON 1445, the EFSA GMO Panel concludes that it is highly unlikely that cotton MON 531 × MON 1445 will contribute to the environmental prevalence of *npt*II genes. Potential interactions of cotton MON 531 ×

---

[1] On request from the Competent Authority of the United Kingdom for an application (EFSA-GMO-UK-2005-09) submitted by Monsanto, Question No EFSA-Q-2005-012, adopted on 8 March 2012.

[2] On request from the European Commission for an application (EFSA-GMO-RX-MON531×MON1445) submitted by Monsanto, Question No EFSA-Q-2007-152, adopted on 8 March 2012.

[3] Panel members: Hans Christer Andersson, Salvatore Arpaia, Detlef Bartsch, Josep Casacuberta, Howard Davies, Patrick du Jardin, Gerhard Flachowsky, Lieve Herman, Huw Jones, Sirpa Kärenlampi, Jozsef Kiss, Gijs Kleter, Harry Kuiper, Antoine Messéan, Kaare Magne Nielsen, Joe Perry, Annette Pöting, Jeremy Sweet, Christoph Tebbe, Atte Johannes von Wright, and Jean-Michel Wal. Correspondence: gmo@efsa.europa.eu

[4] Acknowledgement: The Panel wishes to thank the members of the Working Groups on Molecular Characterisation, Food and Feed and Environment for the preparatory work on this scientific opinion; Boet Glandorf and Willem Seinen as external experts; and the EFSA's staff members Zoltán Divéki, Christina Ehlert and Andrea Germini for the support provided to this scientific opinion.

Scientific Opinion on applications EFSA-GMO-UK-2005-09 and EFSA-GMO-RX-MON531×MON1445 for the placing on the market food and feed produced from or containing ingredients produced from insect-resistant and herbicide-tolerant genetically modified cotton MON 531 × MON 1445; and for the renewal of authorisation of cotton MON 531 × MON 1445 as an existing product, both under Regulation (EC) No 1829/2003 from Monsanto. The EFSA Journal (2012);10(3):2608. doi:10.2903/j.efsa.2012.2608. Available online: www.efsa.europa.eu/efsajournal

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.

KEY WORDS

GMO, cotton, MON 531 × MON 1445, Bollgard® with Roundup Ready®, insect resistance, herbicide tolerance, risk assessment, Regulation (EC) No 1829/2003

SUMMARY

Following a request from the Competent Authority of the United Kingdom and from the European Commission (EC), the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) was asked to deliver a scientific opinion on applications EFSA-GMO-UK-2005-09 and EFSA-GMO-RX-MON531×MON1445, respectively, both submitted by Monsanto under Regulation (EC) No 1829/2003.[5] Whereas application EFSA-GMO-UK-2005-09 is for the placing on the market of food and feed products derived from cotton MON 531 × MON 1445, EFSA-GMO-RX-MON531×MON1445 is for renewal of the authorisation for continued marketing of:

- food additives produced from cotton MON 531 × MON 1445, authorised under 89/107/EEC;[6]
- feed produced from cotton MON 531 × MON 1445 (feed materials and feed additives), authorised under Directive 70/524/EEC.[7]

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

Since both EFSA-GMO-UK-2005-09 and EFSA-GMO-RX-MON531×MON1445 cover products derived from cotton MON 531 × MON 1445 that do not contain viable plant parts, the EFSA GMO Panel provides a single scientific opinion, valid for both applications.

The EFSA GMO Panel assessed cotton MON 531 × MON 1445 with reference to the intended uses and appropriate principles described in its Guidance Documents for the risk assessment of GM plants and derived food and feed (EFSA, 2006a), for the risk assessment of GM plants containing stacked transformation events (EFSA, 2007) and for renewal of authorisations of existing GMO products lawfully placed on the market (EFSA, 2006b). In delivering its Scientific Opinion, the EFSA GMO Panel considered applications EFSA-GMO-UK-2005-09 and EFSA-GMO-RX-MON531×MON1445, additional information submitted by the applicant on request of the Panel, the scientific comments submitted by Member States and relevant scientific publications. Further information from applications for the renewal of the authorisation for continued marketing of single cotton events MON 531 and MON 1445 was also taken into account. In accordance with its Guidance Document for renewal of authorisations of existing GMO products (EFSA, 2006b), the EFSA GMO Panel took into account the new information, experience and data on cotton MON 531 × MON 1445 that 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 was evaluated with respect to potential toxicity, allergenicity and nutritional quality. An assessment was made of environmental impacts and the necessity for a post-market environmental monitoring plan.

The single cotton events MON 531 and MON 1445 were the subject of separate earlier risk assessment evaluations by the EFSA GMO Panel. The Panel concluded that they are unlikely to have any adverse effect on human and animal health or the environment, in the context of their intended uses (EFSA, 2011a and b). No new genes, in addition to those occurring in cotton MON 531 and MON 1445, have been introduced in cotton MON 531 × MON 1445. Cotton MON 531 × MON 1445 was produced by conventional crossing of the single cotton events to combine in the same stack resistance against certain lepidopteran target pests and tolerance to glyphosate-based herbicides.

[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.

[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.

Molecular analysis confirmed that the MON 531 and MON 1445 functional inserts are present and that their structures are retained in cotton MON 531 × MON 1445. Result of the bioinformatic analyses of the flanking sequences and the open reading frames spanning the insert-genomic DNA junctions did not reveal safety issues. The overall levels of the Cry1Ac and CP4 EPSPS proteins were comparable to those of the corresponding single cotton events MON 531 and MON 1445.

The comparative analyses indicated that no biologically relevant differences were identified in the compositional, agronomic and phenotypic characteristics of cotton MON 531 × MON 1445 compared with its conventional counterpart and that the composition of cotton MON 531 × MON 1445 fell within the range of non-GM cotton varieties, except for expressing the CP4 EPSPS, Cry1Ac and NPTII proteins. The safety assessment identified no issues regarding toxicity and allergenicity of cotton MON 531 × MON 1445. A feeding study in catfish confirmed that toasted cottonseed meal from MON 531 × MON 1445 was as nutritious as toasted cottonseed meal from its conventional counterpart and four commercial non-GM cotton varieties.

According to the information provided by the applicant, food and feed products produced from cotton MON 531 × MON 1445 have been consumed without reports of adverse effects since they were placed on the market in the European Union.

The scope of applications EFSA-GMO-UK-2005-09 and EFSA-GMO-RX-MON531×MON1445 only covers only food and feed products produced from cotton MON 531 × MON 1445 that contain no viable plant parts. Therefore, there are no requirements for scientific information on the environmental risks associated with the accidental release or cultivation of cotton MON 531 × MON 1445. No risk arising from a horizontal gene transfer of the CP4 *epsps* and *cry1Ac* genes from cotton MON 531 × MON 1445 to bacteria was identified. There is sequence similarity between parts of the functional insert flanking the *npt*II gene and naturally occurring bacterial plasmid sequences. Cotton MON 531 × 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 531 × MON 1445 in bacteria. However, considering the overall limited occurrence of horizontal transfer of DNA in plant material to bacteria, and the very low exposure to DNA from cotton MON 531 × MON 1445, the EFSA GMO Panel concludes that it is highly unlikely that cotton MON 531 × MON 1445 will contribute to the environmental prevalence of *npt*II genes. The assessment of horizontal gene transfer from cotton MON 531 × MON 1445 to bacteria does therefore 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 531 × MON 1445 with non-target organisms and the abiotic environment were not considered to be an issue owing to the low level of exposure. A post-market environmental monitoring plan for cotton MON 531 × MON 1445 is not required.

In conclusion, the EFSA GMO Panel considers that information available for cotton MON 531 × MON 1445 addresses the questions raised by the Member States and that MON 531 × MON 1445-derived products are as safe as products derived from the conventional counterpart in the context of their intended uses.

TABLE OF CONTENTS

BACKGROUND

On 6 January 2005, the European Food Safety Authority (EFSA) received from the United Kingdom Competent Authority an application (EFSA-GMO-UK-2005-09) for authorisation of genetically modified (GM) cotton MON 531 × MON 1445 (Unique Identifier MON-ØØ531-6 × MON-Ø1445-2) submitted by Monsanto within the framework of Regulation (EC) No 1829/2003 on GM food and feed. After receiving the application EFSA-GMO-UK-2005-09, and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed the Member States and the European Commission (EC) and made the summary of the application publicly available on the EFSA website.[8] 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 12 July 2005, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

On 29 June 2007, EFSA received from the EC an application (EFSA-GMO-RX-MON531×MON1445) submitted under Regulation (EC) No 1829/2003 for renewal of the authorisation of food additives produced from cotton MON 531 × MON 1445 and feed produced from cotton MON 531 × MON 1445 (feed materials and feed additives).

The scope of the renewal application, as described in the Community Register,[9] covers the continued marketing of:

- food additives produced from cotton MON 531 × 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;

- feed produced from cotton MON 531 × 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.

After receiving the renewal application EFSA-GMO-RX-MON531×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 EC and made the summary of this application publicly available on the EFSA website.[10] 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 12 March 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 applications EFSA-GMO-UK-2005-09 and EFSA-GMO-RX-MON531×MON1445 available to Member States and the EC, and consulted nominated risk assessment bodies of Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC,[11] to request their scientific opinion. The Member State bodies had 3 months after the date of receipt of the valid application (until 12 October 2005 and 12 June 2008, respectively) within which to make their opinion known.

The scope of applications EFSA-GMO-UK-2005-09 and EFSA-GMO-RX-MON531×MON1445 covers the stacked event in the cotton species *Gossypium hirsutum* L. and *G. barbadense* L.

[8] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2005-012
[9] http://ec.europa.eu/food/dyna/gm_register/gm_register_auth.cfm?pr_id=10
[10] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2007-152
[11] 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.

The Scientific Panel on Genetically Modified Organisms of EFSA (EFSA GMO Panel) carried out an evaluation of the risk assessment of the applications on cotton MON 531 × MON 1445 in accordance with the appropriate principles described in its guidance documents for the risk assessment of GM plants and derived food and feed (EFSA, 2006a), for the risk assessment of GM plants containing stacked transformation events (EFSA, 2007) and for renewal of authorisations of existing GMO products lawfully placed on the market (EFSA, 2006b) were taken into consideration. In addition, the scientific comments of Member States, the additional information provided by the applicant and relevant scientific publications were taken into consideration. Further information from applications for the renewal of the authorisation for continued marketing of single cotton events MON 531 and MON 1445 was also taken into account.

For EFSA-GMO-UK-2005-09, the EFSA GMO Panel requested additional information from the applicant on 31 January 2006, 24 June 2008, 12 September 2008, 23 October 2008, 30 January 2009, 7 April 2009, 28 April 2009, 24 July 2009, 18 September 2009, 27 October 2009, 4 February 2010 and on 27 May 2010. The applicant provided the requested information on 16 August 2007, 11 December 2007, 4 July 2008, 18 November 2008, 9 December 2009, 17 March 2010 and 14 September 2010.

For EFSA-GMO-RX-MON531×MON1445, the EFSA GMO Panel requested additional information from the applicant on 13 March 2008, 24 June 2008, 9 September 2008, 23 October 2008, 30 January 2009, 7 April 2009, 28 April 2009, 24 July 2009, 18 September 2009, 27 October 2009, 4 February 2010 and on 27 May 2010. The applicant provided the requested information on 4 July 2008, 14 November 2008 and 14 September 2010.

In giving its scientific opinion on cotton MON 531 × MON 1445 to the EC, 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 6 months from the acknowledgement 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, 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 respective overall opinions 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 531 × MON 1445 (Unique Identifier: MON-ØØ531-6 × MON-Ø1445-2) in the context of applications EFSA-GMO-UK-2005-09 and EFSA-GMO-RX-MON531×MON1445. Whereas application EFSA-GMO-UK-2005-09 is for the placing on the market of food and feed products derived from cotton MON 531 × MON 1445, the scope of EFSA-GMO-RX-MON531×MON1445 covers the renewal of authorisation of (1) food additives produced from cotton MON 531 × 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 (2) feed produced from cotton MON 531 × 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 GM cotton MON 531 × MON 1445 (Unique Identifier MON-ØØ531-6 × MON-Ø1445-2) was evaluated with reference to its intended uses, taking account of the appropriate principles described in the guidance documents of the EFSA GMO Panel for the risk assessment of GM plants and derived food and feed (EFSA, 2006a), for the risk assessment of GM plants containing stacked transformation events (EFSA, 2007) and for the renewal of authorisations of existing GMO products lawfully placed on the market (EFSA, 2006b). The evaluation of the risk assessment presented here is based on the information provided in the application, as well as additional information from the applicant, scientific comments submitted by the Member States and relevant scientific publications.

2. Issues raised by the Member States

The scientific issues raised by the Member States are addressed in Annex G of the EFSA overall opinion and have been considered in this scientific opinion.[12,13]

3. Molecular characterisation

3.1. Evaluation of relevant scientific data

3.1.1. Method of production of cotton MON 531 × MON 1445

Conventional breeding methods were used to develop cotton MON 531 × MON 1445, and no new genetic modification was involved.[14] The inserts that are present in cotton MON 531 × MON 1445 were derived from cotton lines containing two independent events: MON 531 and MON 1445. The GM cottons MON 531 and MON 1445 were the subjects of earlier safety evaluations (EFSA, 2011a, 2011b). Cotton MON 531 × MON 1445 combines resistance to certain lepidopteran pests with tolerance to glyphosate-based herbicides.

3.1.2. Summary of the evaluation of the single events

3.1.2.1. MON 531

Cotton MON 531 was developed through *Agrobacterium tumefaciens* (renamed as *Rhizobium radiobacter*)-mediated transformation and includes two expression cassettes. One of the cassettes expresses a synthetic *cry1A* gene under the control of an enhanced *Cauliflower mosaic virus* (CaMV) 35S promoter to confer resistance to certain lepidopteran pests. The synthetic gene codes for a Cry1Ac protein variant with 99.4 % amino acid sequence identity to the Cry1Ac of *Bacillus thuringiensis*. The other cassette expresses a neomycin phosphotransferase II (*npt*II) gene under the control of the CaMV 35S promoter. Expression of the *npt*II gene allowed for selection of the transformed plant cells with kanamycin. Cotton MON 531 also carries the *aad*A gene from transposon Tn*7*, which is not expressed in MON 531 but was used as a marker gene during product development. Other parts of the transformation vector, such as the *ori*V origin of replication, were also inserted into cotton MON 531.

Molecular characterisation data established that cotton MON 531 contains two separate transfer DNA (T-DNA) elements. The functional insert contains the Cry1Ac cassette, the *aad*A gene, the NPTII cassette and the *ori*V origin of replication. Adjacent to this insert, a fragment containing the 3' portion of the Cry1Ac cassette was inserted. Additionally, a non-functional fragment containing a portion of

[12] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2005-012
[13] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2007-152
[14] Technical Dossier/Section C

the 7S 3' transcriptional termination sequence of the Cry1Ac cassette was inserted into cotton MON 531 at a separate locus.

Bioinformatic analyses of the 5' and 3' flanking regions of the inserts did not reveal disruption of known cotton genes or the creation of novel open reading frames (ORFs) that would show significant similarity to known allergens or toxins. The bioinformatic analyses support the conclusion that, even in the unlikely event that any of the new ORFs at the junctions were translated, they would not raise a safety issue.

The stability of the genetic modification has been demonstrated over several generations by Southern analysis, and the expected inheritance was observed for the Cry1Ac protein over several generations, indicating the presence of a single genetic locus with Mendelian segregation. The insect resistance phenotype of MON 531 has been demonstrated under field conditions on a commercial scale since 1996, e.g. in the USA and Australia. A more detailed evaluation of the MON 531 event can be found in a previous EFSA opinion (EFSA, 2011a).

3.1.2.2. MON 1445

Cotton MON 1445 was developed through *A. tumefaciens* (renamed as *R. radiobacter*)-mediated transformation and includes two expression cassettes. One of the cassettes expresses a gene encoding the 5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium* sp. strain CP4 (CP4 EPSPS), which confers tolerance to glyphosate-based herbicides, under the control of the modified *Figwort mosaic virus* promoter. The other cassette expresses a *npt*II gene under the control of the CaMV 35S promoter. Expression of the *npt*II gene allowed for selection of the transformed plant cells with kanamycin. Cotton MON 1445 also carries the *aad*A gene from transposon Tn*7*, which is not expressed in MON 1445 but was used as a marker gene during product development. Other parts of the transformation vector, such as part of the *ori*V origin of replication, were also inserted into cotton MON 1445.

Molecular characterisation data established that cotton MON 1445 contains a single T-DNA insert consisting of part of the *ori*V origin of replication, *npt*II expression cassette, *aad*A gene and the CP4 *epsps* expression cassette.

Bioinformatic analyses of the 5' and 3' flanking regions did not reveal disruption of known cotton genes or the creation of novel ORFs that would show significant similarity to known allergens or toxins. The bioinformatic analyses support the conclusion that, even in the unlikely event that any of the new ORFs at the junctions were translated, they would not raise a safety issue.

The stability of the genetic modification was demonstrated over several generations by Southern analysis, and the expected inheritance was observed over two generations, based on glyphosate tolerance of the progeny, indicating the presence of a single genetic locus with Mendelian segregation. The herbicide-tolerant 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. A more detailed evaluation of the MON 1445 event can be found in a previous EFSA opinion (EFSA, 2011b).

3.1.2.3. Antibiotic resistance marker genes in MON 531 and MON 1445

Two antibiotic resistance marker genes are present in both cotton MON 531 and MON 1445 as a consequence of the genetic modification process. The transfer of antibiotic resistance genes from GM plants to bacteria has not been shown to occur either under natural conditions or in the laboratory in the absence of nucleotide sequence identity in the recipient bacterial cell (EFSA, 2009). 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. In MON 531 and 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 event. No cryptic targets of homologous recombination were identified. The possibility of double homologous recombination was investigated. For MON 531, two alignment pairs were found showing in the same plasmid the presence of an *aad*A gene and an *ori*V sequence with sufficient similarity to allow homologous recombination. Due to the presence of similar genetic elements, one of these alignment pairs was also identified for MON 1445. The hits were found in plasmids from two bacteria, one isolated from activated sludge and the other from soil. 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 sequence of the two bacteria.

3.1.3. Transgene constructs in MON 531 × MON 1445

The integrity of the individual inserts present in this cotton was investigated using Southern analyses.[15] This involved the use of DNA probes specific for MON 531 and MON 1445 inserts and the use of restriction enzymes that allowed the structures of the inserts, including the junction regions, to be determined within the stack. The predicted DNA hybridisation patterns from each single event were retained in the MON 531 × MON 1445 stack, demonstrating that integrity of the inserts was maintained.

3.1.4. Information on the expression of the insert

The levels of Cry1Ac, CP4 EPSPS and NPTII in cotton MON 531 × MON 1445 were analysed by enzyme-linked immunosorbent assay (ELISA) in three different genetic backgrounds.[16] Tissue samples for analysis were collected from four field trials conducted in the USA during 1998. The trials were located in major cotton-growing regions of USA and provided a variety of environmental conditions. Each trial included appropriate comparators (MON 531 and MON 1445 as positive controls, and conventional cotton varieties with a genetic background similar to cotton MON 531 × MON 1445 as negative controls). Young leaf and seed tissues were collected from each site.

The scope of the applications covers food and feed produced from MON 531 × MON 1445, therefore protein expression data related to the seeds are considered most relevant. Table 1 summarises protein expression data for one genetic background and indicates that levels of the Cry1Ac and CP4 EPSPS proteins in the stacked line were comparable to levels in the single events. Comparable data were obtained for other genetic backgrounds. The level of the NPTII protein was higher in the stack than in the single events probably due to the presence of two *npt*II expression cassettes in cotton MON 531 × MON 1445.

Table 1: Summary of protein levels in seeds of cotton MON 531 × MON 1445 in variety DP50 (µg/g dry weight).

Cotton variety DP50	MON 531 × MON 1445	MON 531	MON 1445
Cry1Ac, mean	2.62	2.45	< LOD
range	1.75-3.24	0.84-3.02	
CP4 EPSPS, mean	310	< LOD	255
range	280-339		175-305
NPTII, mean	51.8	11.9	30.4
range	43.6-60.7	11.4-12.1	25.2-42.1

LOD: level of detection

[15] Additional information, July 2008
[16] Technical Dossier / Section D3

3.1.5. Inheritance and stability of inserted DNA

The genetic stability of the inserted DNA in events MON 1445 and MON 531 was demonstrated previously (EFSA, 2011a, 2011b). In cotton MON 531 × MON 1445 the two inserts were combined. Genetic stability of the inserts in the stacked line was demonstrated over multiple generations.[17] The Southern data show that the integrity of the inserts present in the single events is retained in MON 531 × MON 1445. Phenotypic stability of the insect resistance and herbicide tolerance traits was demonstrated under commercial conditions from 1997 onwards.

3.2. Conclusion

As conventional breeding methods were used in the production of cotton MON 531 × MON 1445, no additional genetic modification was involved. Southern analyses demonstrated that the integrity of the functional inserts in the MON 531 and MON 1445 events was retained in cotton MON 531 × MON 1445. Genetic and phenotypic stability of the inserts was demonstrated. The levels of Cry1Ac and CP4 EPSPS proteins in the seeds of cotton MON 531 × MON 1445 were comparable to those of the single events. The EFSA GMO Panel concludes that the molecular characterisation does not indicate safety issues. The potential identified for horizontal transfer of the *npt*II gene by homologous recombination is further evaluated in Section 6.1.2.

4. Comparative analysis

4.1. Evaluation of relevant scientific data

4.1.1. Summary of the previous evaluation of the single cotton events

4.1.1.1. Cotton MON 531

Cottonseed from event MON 531 and its conventional counterpart Coker312 were harvested from field trials in the USA in 1992 and 1993 and used in comparative compositional analysis. Additional compositional data were available for cottonseed harvested in the USA in 1999. As the cotton MON 531 event in the latter field trials had been bred into the DP5415 genetic background, the conventional counterpart in the field trial in 1999 was DP5415 instead of Coker312. In addition, this field trial included 11 commercial non-GM reference lines. The compositional data of these reference lines were used to develop a 99 % tolerance interval for each compositional parameter studied. Historical and literature values were also provided for these parameters in cottonseed.

Unprocessed cottonseed, toasted meal and refined cottonseed oil produced from pooled samples of cottonseed harvested in the USA in 1993 were analysed for their composition. The harvested material and produced products were analysed for key nutrients, anti-nutrients and toxicants as defined by OECD (2009). Vitamin E (α-tocopherol) was reported for refined cottonseed oil produced in 1993 and crude fibre in the cottonseed harvested in 1999.

Agronomic and phenotypic characteristics of cotton MON 531, its conventional counterparts and reference varieties were analysed in field trials in the USA in 1998 and in 1999. In 1998 the MON 531 event was tested in three genetic backgrounds (DP50, DP5690 and DP5415), whereas in 1999 it was tested in the DP5415 background. The agronomic and phenotypic parameters studied were related to seed and plant development, disease and pest susceptibility, reproduction and yield.

Analyses carried out on cotton MON 531, its conventional counterpart and other non-GM cotton varieties indicated that no biologically relevant differences were identified in the compositional, phenotypic and agronomic characteristics of cotton MON 531 in comparison with its conventional counterparts. Furthermore its composition fell within the range of non-GM cotton varieties, except for expressing the introduced trait (EFSA, 2011a).

[17] Technical Dossier / Section D2

4.1.1.2. Cotton MON 1445

Seeds of cotton MON 1445 and its conventional counterpart Coker312 were harvested from field trials performed in the USA in 1993 and 1994. The field trial performed in 1994 included cotton MON 1445 plots treated with the specific herbicide glyphosate as well as plots treated with conventional herbicides. Additional compositional data were available from cottonseed of MON 1445 bred into the DP5415 genetic background and harvested from field trials in the USA in 1999. In addition, these field trials included the conventional counterpart DP5415 and 11 commercial non-GM reference lines. The harvested material and produced products were analysed for key nutrients, anti-nutrients and toxicants as defined by OECD (2009). Vitamin E (α-tocopherol) was reported for refined cottonseed oil produced in 1993 and crude fibre in the cottonseed harvested in 1994 and 1999. Cottonseed harvested in 1993 was also processed into toasted meal and refined cottonseed oil and analysed for its composition.

The agronomic and phenotypic characteristics of cotton MON 1445, its conventional counterparts and reference varieties were analysed in field trials conducted under normal agronomic conditions in the USA in 1998 and 1999. In 1998 the MON 1445 event was tested in three genetic backgrounds (DP50, DP5690 and DP5415), whereas in 1999 it was tested in the DP5415 background. The agronomic and phenotypic parameters studied were related to seed and plant development, disease and pest susceptibility, reproduction and yield.

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 or agronomic characteristics of cotton MON 1445 in comparison with its conventional counterpart, and its composition fell within the range of non-GM cotton varieties, except for expressing the introduced trait (EFSA, 2011b).

4.1.2. Choice of comparator and production of material for the compositional assessment[18]

The composition, agronomic and phenotypic characteristics of cotton MON 531 × MON 1445 were studied in field trials performed in the USA, Spain and Argentina in 1998 and/or 1999.

The 1998 non-replicated field trials at four locations in the USA were performed with the stacked cotton MON 531 × MON 1445 in three different genetic backgrounds (the Delta and Pine varieties DP50, DP5690 and DP5415). These varieties, DP50, DP5690 and DP5415, were used at each location as the conventional counterparts. The field trials also included the parental GM lines MON 531 and MON 1445 in the three different genetic backgrounds mentioned. The field trials in the USA included commercial cotton lines as reference materials to illustrate the natural variation in cotton of the parameters studied; for this purpose 11 non-GM reference lines were included in 1998 and four in 1999. Non-replicated field trials were performed in 1998 in Spain at three locations with cotton MON 531 × MON 1445 and the conventional counterpart in the DP5690 genetic background. In 1999, field trials were conducted at the same four sites in the USA as for the 1998 study, but using only cotton MON 531 × MON 1445 and the comparator in the DP5415 genetic background; cotton MON 531 × MON 1445 and the control cotton line DP5415 were planted in a randomised complete block design with four replications. A similar field trial was conducted in 1999 at two locations in Argentina with cotton MON 531 × MON 1445 in the DP50 genetic background. The EFSA GMO Panel is of the opinion that the DP varieties used as non-transgenic control lines constitute appropriate conventional counterparts. Whereas cotton MON 531 × MON 1445 was treated with glyphosate in the USA, in Spain and Argentina the cotton MON 531 × MON 1445 was not sprayed with glyphosate. The harvested cottonseed was ginned and acid delinted before compositional analysis.

[18] Technical dossier / Section D7.2 and Additional information, December 2009

4.1.3. Compositional analysis[19]

Compositional analysis was performed on cottonseed obtained from the field trials performed during 1998 and 1999. In the analysis of the 1998 field trial data from USA and Spain the component values for the stacked MON 531 × MON 1445 cotton were compared with those of the corresponding single trait parental lines MON 531 and MON 1445 and the conventional counterpart for each genetic background tested. Additional compositional data were obtained from DP genotypes grown in the field trials in the US and Spain. However, the design of these two studies did not include any replication; hence these studies were used only as complementary information for the compositional analyses of cotton MON 531 × MON 1445.

In the analysis of the 1999 field trial data from USA and Argentina, cotton MON 531 × MON 1445 lines were compared with both single trait parental lines MON 531 and MON 1445 and their conventional counterpart across all locations as well as within each location. Commercial reference line data were used in the USA study to develop tolerance intervals for each parameter measured.

The analysed compounds were in line with the suggestions of the OECD (2009), apart from vitamin E which was not analysed; the analyses included proximates, amino acids, fatty acids, minerals (Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn), cyclopropenoid fatty acids and toxicants (total gossypol in 1998 and total and free gossypol in 1999).

The compositional comparison of cottonseed of MON 531 × MON 1445 and its conventional counterpart harvested from field trials in the USA in 1999 revealed 28 statistically significant differences out of the 245 comparisons made. Of these significant differences, only four were observed in the across-sites analysis. Thus, the copper, iron and magnesium values were higher and the potassium level lower in the conventional counterpart than in cotton MON 531 × MON 1445. The other 24 differences were observed for different constituents at individual field trial sites. For all analytes the measured levels in cotton MON 531 × MON 1445 fell within the 99 % tolerance interval established from the commercial cotton varieties planted in the same field trials. In the compositional assessment of cottonseed from the field trials in Argentina in 1999 no differences in mineral levels, as observed in the 1999 field trials in the USA, were found, neither in the across-location analysis nor in the within-location analysis. Twelve out of the 144 comparisons between cotton MON 531 × MON 1445 and its conventional counterpart showed significant differences but only three of these twelve significant differences were observed in the combined site analysis. These differences concerned small increases in the level of glutamic acid and linoleic acid and a small decrease in linolenic/γ-linolenic acid level.

In the field trial in Spain in 1998, cotton MON 531 × MON 1445 was planted together with its parental lines and its conventional counterpart DP5690. In the compositional comparison of the cottonseed statistically significant differences were observed between cotton MON 531 × MON 1445 and the parental lines. These differences were small and in all cases the mean values for the individual compounds measured in seeds from the stacked cotton MON 531 × MON 1445 and the parental lines were well within the ranges of these parameters in the reference varieties. However, one parameter, the total gossypol content in seeds was significantly higher in cotton MON 531 × MON 1445 than in both parental cotton lines, the total gossypol content being in the upper range for the reference cotton varieties. However, it should be noted that no increase in total gossypol content of cotton MON 531 × MON 1445 was observed when compared with the appropriate conventional counterpart in the material from the US field trials, irrespective of the genetic background in which the MON 531 × MON 1445 events appeared.

On the basis of the compositional assessment of cottonseed of MON 531 × MON 1445, its conventional counterparts, and the parental GM events MON 531 and MON 1445 obtained from field trials in the USA, Spain and Argentina, the EFSA GMO Panel concludes that statistically significant

[19] Technical Dossier / Section D7.1 and Additional information, December 2009

differences observed were small and did not occur consistently over the two seasons and the geographical sites. Furthermore, measured levels fell within the natural ranges of commercial reference cotton varieties when included in the field trials. Therefore, the Panel did not consider these differences to have biological relevance or to be related to the genetic modification. The Panel concludes that no biologically relevant differences were identified in the compositional characteristics of cotton MON 531 × MON 1445 in comparison with its conventional counterpart and that its composition fell within the range of non-GM cotton varieties, except for expressing the Cry1Ac, CP4 EPSPS and NPTII proteins.

4.1.4. Agronomic traits and GM phenotype[20]

Phenotypic and agronomic characteristics of cotton MON 531 × MON 1445, its conventional counterparts and the parental cotton events MON 531 and MON 1445 were studied during field trials in the USA in 1998. These field trials were performed with the events bred into three genetic backgrounds (DP50, DP5690 and DP5415). Measurements of phenotypic and agronomic characteristics included indicators of growth and development and were height, flowering dates and boll counts, germination, emergence, vigor, start of flowering, yield, disease incidence and insect damage. No significant differences were observed between cotton MON 531 × MON 1445 and its conventional counterpart for any of the three genetic backgrounds. There was also no difference between cotton MON 531 × MON 1445 and the two parental cotton events MON 531 and MON 1445. It is concluded that crossing insect-resistant cotton MON 531 with glyphosate-tolerant cotton MON 1445 to produce the stacked cotton MON 531 × MON 1445 did not result in any consistent changes in phenotypic and agronomic characteristics compared with its conventional counterpart and the parental cotton lines.

4.2. Conclusion

Compositional analysis of key nutrients, anti-nutrients, and toxins in cottonseed was carried out on cotton grown on three continents and harvested during two growing seasons. A number of statistically significant differences were observed in seeds from cotton MON 531 × MON 1445 compared with seeds from its conventional counterparts as well as the single parental events MON 531 and MON 1445. Although differences were observed, they were inconsistent (i.e. not in each year and/or location) and always fell within the background ranges in the level of studied constituents defined by cottonseed from commercial varieties. No significant differences were observed in phenotypic and agronomic characteristics compared with its conventional counterpart and the parental cotton lines. The EFSA GMO Panel therefore concludes that no biologically relevant differences were identified in the compositional, agronomic or phenotypic characteristics of cotton MON 531 × MON 1445 compared with its conventional counterpart and that the composition of cotton MON 531 × MON 1445 fell within the range of non-GM cotton varieties, except for expressing the CP4 EPSPS, Cry1Ac and NPTII proteins. Based on the assessment of the data available the EFSA GMO Panel is of the opinion that crossing cotton MON 531 and cotton MON 1445 to produce cotton MON 531 × MON 1445 does not result in interactions that cause compositional, agronomic or phenotypic changes that would raise a safety issue.

5. Food/Feed safety assessment

5.1. Evaluation of relevant scientific data

5.1.1. Summary of the previous evaluation of the single events

5.1.1.1. Cotton MON 531

Cotton MON 531 expresses the Cry1Ac and NPTII proteins. *Escherichia coli*-produced Cry1Ac and NPTII proteins were used for toxicity studies after it had been demonstrated experimentally that they

[20] Technical Dossier / Section D7.4

were equivalent to those expressed in cotton MON 531. The newly expressed Cry1Ac and NPTII proteins induced no adverse effects in acute oral toxicity studies in mice at high dose levels and they are rapidly degraded in simulated gastric fluid and inactivated during processing to toasted cottonseed meal. The amino acid sequence of the newly expressed Cry1Ac and NPTII proteins did not show any significant similarity with the amino acid sequences of known toxins or allergens. The EFSA GMO Panel concluded that cotton MON 531 is as safe and nutritious as its conventional counterpart, and that the overall allergenicity of the whole plant is not changed. Cotton MON 531 and its derived products are unlikely to have any adverse effects on human and animal health in the context of their intended uses (EFSA, 2011a).

5.1.1.2. Cotton MON 1445

Cotton MON 1445 expresses the CP4 EPSPS and NPTII proteins (EFSA, 2011b). E. *coli*-produced CP4 EPSPS and NPTII proteins were used for toxicity studies after it had been demonstrated experimentally that they were equivalent to those expressed in cotton MON 1445. The newly expressed CP4 EPSPS and NPTII proteins induced no adverse effects in acute oral toxicity studies in mice at high dose levels and they are rapidly degraded in simulated gastric fluid and inactivated during processing to toasted cottonseed meal. The amino acid sequence of the newly expressed CP4 EPSPS and NPTII proteins did not show any significant similarity with the amino acid sequences of known toxins or allergens. Nutritional data comprising a target animal feeding study on catfish fed a diet containing 20 % processed cottonseed meal for 10 weeks indicate that cotton MON 1445 is nutritionally equivalent to its conventional counterpart. The EFSA GMO Panel concluded that cotton MON 1445 is as safe and nutritious as its conventional counterpart and that the overall allergenicity of the whole plant is not changed. Cotton MON 1445 and its derived products are unlikely to have any adverse effects on human and animal health in the context of their intended uses (EFSA, 2011b).

5.1.2. Product description and intended use[21]

The scope of application EFSA-GMO-UK-2005-09 covers (1) food produced or containing ingredients produced from cotton from MON 531 × MON 1445 and (2) feed produced from MON 531 × MON 1445. The scope of application EFSA-GMO-RX-MON531×MON1445 is renewal of the authorisation for continued marketing of (1) food additives produced from cotton MON 531 × MON 1445 and (2) feed produced from cotton MON 531 × MON 1445 (feed materials and feed additives). Thus, the possible uses of cotton MON 531 × MON 1445 include the production of refined oil from seeds, production of cellulose from linters as food or food ingredients and use of cottonseed meal and hulls in animal feed.

The genetic modification of cotton MON 531 × MON 1445 is intended to improve agronomic performance only, and is not intended to influence the nutritional properties, processing characteristics or overall use of cotton as a crop.

Cotton MON 531 × MON 1445 was first cultivated in the USA in 1997. By 2006 cotton MON 531 × MON 1445 was also cultivated in Mexico and South Africa. It is not commercially produced in any of the 27 countries of the EU. Globally, the production of cotton MON 531 × MON 1445 has grown rapidly since its introduction and in 2006 reached adoption rates exceeding 50 % of the total cotton production area in the USA and 30 % in Mexico and South Africa. In recent years, production of cotton MON 531 × MON 1445 has levelled off or declined, being replaced by stacked cotton event MON 15985 × MON 1445.

Based on the data for imports of cottonseed oil and cottonseed meal from cotton MON 531 × MON 1445-producing countries into the 27 countries of the EU over the years 2003-2005, the applicant has calculated that an insignificant amount of cottonseed oil and around 0.33 % of cottonseed meal used in the EU might be derived from cotton MON 531 × MON 1445. It should be noted, however, that these

[21] Technical dossier / Section D7.5 and D7.7

calculations are based on several assumptions. Because operators in the food and feed chain in some Member States of the EU have made efforts to preferentially source non-GM products, the actual consumption of products derived from cotton MON 531 × MON 1445 in food and feed may vary between Member States.

Animal feed is the major end use of cottonseed meal. Compared with the total amount of mixed feed produced in the EU (approximately 150 million tonnes per year) and based on recent years' import data, the portion of imported cotton seed meal represents less then 0.2 % of total mixed feed.

5.1.3. Effect of processing[22]

As no biologically relevant differences were identified in the compositional characteristics of cotton MON 531 × MON 1445 in comparison with its conventional counterpart, except for the newly expressed proteins (see section 4.1.2), the effect of processing on cotton MON 531 × MON 1445 was not expected to result in products any different from conventional cotton varieties. The effect of processing on the levels of the newly expressed proteins (Cry1Ac, CP4 EPSPS and NPTII) in cottonseed-derived products (processed cottonseed meals, oil and linters) was studied in the parental cottons MON 531 and MON 1445 lines using enzymatic activity assays and Western blot analysis but not in cotton MON 531 × MON 1445. In the processed cottonseed products derived from cotton MON 531 the Cry1Ac protein was not detected in processed cottonseed meal or in processed linters (see Section 4.1.1). More than 96.7 % of the NTPII protein in seeds of cotton MON 531 lost its detectability by Western blot analysis during processing to toasted cottonseed meal. CP4 EPSPS enzymatic activity could be demonstrated in seed and full-fat flour but not in toasted meal from cotton MON 1445. In combed lint (mechanically cleaned raw lint) Western blot analysis detected 0.5 μg CP4 EPSPS/g protein, but no protein was found in processed linter (limit of detection = 0.1 μg/g of protein). Studies were also provided indicating that no proteins could be found in refined cottonseed oil, whether produced from conventional cottonseed or GM cottonseed. It can be assumed that in products derived from cottonseed MON 531 × MON 1445 the newly expressed proteins are affected by processing in the same way as they are in the parental lines described above.

5.1.4. Toxicology[23]

5.1.4.1. Toxicological assessment of expressed novel proteins in cotton MON 531 × MON 1445

No new genes in addition to those occurring in the parental cotton varieties have been introduced in cotton MON 531 × MON 1445. The Cry1Ac, CP4 EPSPS and NPTII proteins expressed in cotton MON 531 × MON 1445 were recently evaluated for their safety in the context of its parental lines (EFSA, 2011a, 2011b) and no safety issues for humans and animals were identified. Quantification of levels of the Cry1Ac and CP4 EPSPS proteins in seed and leaf tissues of cotton MON 531 × MON 1445, MON 531 and MON 1445 revealed comparable expression levels in the stacked event and the parental events. The level of the NPTII protein was higher in the stack than in the single events probably due to the presence of two *npt*II expression cassettes in cotton MON 531 × MON 1445.

The EFSA GMO Panel considered all the data available for cotton MON 531 × MON 1445, for the single events, and for the newly expressed proteins Cry1Ac, CP4 EPSPS and NPTII, and concludes that interactions between the single events that might impact on food and feed safety are unlikely.

5.1.4.2. Toxicological assessment of new constituents other than proteins

Compared with conventional cotton varieties, no new constituents other than the Cry1Ac, CP4 EPSPS and NPTII proteins are expressed in cotton MON 531 × MON 1445 and no relevant changes in the composition of cotton MON 531 × MON 1445 were detected in the comparative compositional analysis (see Section 4.1.3).

[22] Technical dossier / Section D7.6
[23] Technical dossier / Section D7.8

5.1.4.3. Toxicological assessment of the whole GM food/feed

Both cottons MON 531 and MON 1445 have previously been found to be as safe for human and animal consumption as their corresponding conventional counterparts and commercial non-GM cotton varieties (EFSA, 2011a, 2011b).

A molecular characterisation of cotton MON 531 × MON 1445 identified no altered stability of the single cotton events (see Section 3.1.5) when these were brought together by crossing. Analysis of the levels of Cry1Ac, CP4 EPSPS and NPTII proteins revealed no relevant change in their levels in cotton MON 531 × MON 1445 compared with the respective single cotton events MON 531 and MON 1445 (see Section 3.2).

As no biologically relevant differences were identified in the compositional characteristics of cotton MON 531 × MON 1445 in comparison with non-GM cotton varieties, except for expressing the Cry1Ac, CP4 EPSPS and NPTII proteins and an assessment found no indication of interaction between the single events that could influence the safety of cotton MON 531 × MON 1445 for humans and animals, the EFSA GMO Panel is of the opinion that no additional animal safety studies are required.

5.1.5. Allergenicity[24]

The strategies used when assessing the potential allergenic risk focus on the characterisation of the source of the recombinant protein, the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons and whether the transformation may have altered the allergenic properties of the modified food.

5.1.5.1. Assessment of allergenicity of the newly expressed proteins

A weight-of-evidence approach is recommended, taking into account all of the information obtained with various test methods, as no single experimental method yields decisive evidence for allergenicity (Codex Alimentarius, 2009; EFSA, 2006a, 2010). Allergenicity of the Cry1Ac, CP4 EPSPS and NPTII proteins has been assessed previously (EFSA, 2011a, 2011b), and it was found that they are unlikely to be allergenic, among others grounds on the basis of their fast degradability and the absence of any significant similarity with known protein allergens. Based on these results, the EFSA GMO Panel considers that the newly expressed proteins are unlikely to be allergenic in the intended conditions of exposure.

5.1.5.2. Assessment of allergenicity of the products derived from the GM plant

The 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, e.g. through qualitative or quantitative modifications of the pattern of expression of endogenous proteins. This issue did 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. Edible oils that are refined, bleached and deodorised do not appear to pose a risk to allergic individuals, as they contain virtually no proteins. In the context of the present application the EFSA GMO Panel considers it unlikely that potential interactions will occur in cotton MON 531 × MON 1445 that might change the allergenicity of the products derived from cotton MON 531 × MON 1445.

[24] Technical dossier / Section D7.9

5.1.6. Nutritional assessment of GM food/feed[25]

Comparative analysis showed that no biologically relevant differences were identified in the compositional characteristics of cotton MON 531 × MON 1445 compared with its conventional counterpart, except for the newly expressed proteins in cotton MON 531 × MON 1445. Therefore, in terms of consumption of cottonseed from MON 531 × MON 1445, the EFSA GMO Panel considers that the nutritional properties are likely to be the same as those of other cottonseed. Apart from these considerations, a feeding study with toasted cottonseed meal in catfish was provided.[26]

The nutritional quality of toasted cottonseed meal derived from cotton MON 531 × MON 1445, its conventional counterpart DP5415 and four conventional cotton varieties was fed to groups of 100 catfish with an initial average weight of 5 g for 8 weeks. The inclusion rate of the cottonseed meal in the diet of the catfish was 20 %. At the end of the study, the average weight of the catfish was similar in the various treatment groups, around 60 g. Also feed conversion efficiency, survivability, fillet moisture, fat and ash concentrations were similar for all treatments, but the protein content of fillets from catfish fed toasted cottonseed meal derived from MON 531 × MON 1445 was significantly lower than that of the conventional counterpart. However, the protein content of fillets was within the range recorded for the control references varieties.

The GMO Panel concludes that the data provided supports the view that diets formulated with cottonseed meal derived from MON 531 × MON 1445 are as nutritious as those formulated with cottonseed meal derived from commercial non-GM cotton varieties.

5.1.7. Post-market monitoring of GM food/feed

The risk assessment concluded that no data have emerged to indicate that processed products derived from MON 531 × MON 1445 are as safe as those derived from its conventional counterpart. In addition, diets formulated with cottonseed meal derived from MON 531 × MON 1445 are as nutritious as those formulated with cottonseed meal derived from commercial non-GM cotton varieties. Therefore, and in line with the Guidance document (EFSA, 2006a), the EFSA GMO Panel is of the opinion that post-market monitoring of the GM food/feed derived from cotton MON 531 × MON 1445 is not necessary.

5.2. Conclusion

The newly expressed proteins were examined previously in other applications and no safety issues were identified. The safety of the Cry1Ac, CP4 EPSPS and NPTII proteins is supported by bioinformatics analysis and investigations on stability, digestibility and toxicity. The potential allergenicity of the Cry1Ac, CP4 EPSPS and NPTII proteins has been assessed, and it was considered unlikely that they would be allergenic. As neither the molecular characterisation nor the compositional analysis of the GM cotton indicated any unintended effects, an alteration in the allergenic properties of GM cottonseed appears to be unlikely. In addition, a catfish feeding study confirmed the nutritional equivalence of cottonseed meal from GM cotton MON 531 × MON 1445 to meal from conventional cottonseed.

Given all the information provided, the EFSA GMO Panel considers that interactions between the single events that might impact on food and feed safety are unlikely. The Panel also noted that the nutritional properties of cottonseed meal derived from cotton MON 531 × MON 1445 are not different from those of commercial cotton varieties. The EFSA GMO Panel concludes that products derived from cotton MON 531 × MON 1445 are as safe and nutritious as their conventional counterparts and that it is unlikely that the overall allergenicity of the whole plant is changed.

[25] Technical dossier / Section D7.10

[26] Technical dossier, Appendix "Evaluation of cottonseed meal derived from Bollgard® × Roundup Ready® cotton and Bollgard II® × Roundup Ready® cotton as feed ingredients for channel catfish"

6. Environmental risk assessment and monitoring plan

6.1. Evaluation of relevant scientific data

The scope of applications EFSA-GMO-UK-2005-09 and EFSA-GMO-RX-MON531×MON1445 is for food and feed products produced from cotton MON 531 × MON 1445; the scope of these applications includes only products produced from cotton MON 531 × MON 1445 that contain no viable plant parts. Considering the intended uses of cotton MON 531 × MON 1445, the environmental risk assessment is concerned with analysing the risk of horizontal gene transfer (HGT) of recombinant genes from food and feed material to bacteria in the gastrointestinal tract of humans and animals, as well as indirect exposure through manure and faeces from animals fed with cotton products from cotton MON 531 × MON 1445. There are no requirements for scientific information on environmental safety assessment of accidental release or cultivation of cotton MON 531 × MON 1445.

6.1.1. Evaluation of the single events

In previous scientific opinions, the EFSA GMO Panel was of the opinion that products derived from the single cotton events MON 531 and MON 1445 are as safe as products derived from the conventional counterpart in the context of their intended uses (EFSA, 2011a, 2011b).

6.1.2. Environmental risk assessment

6.1.2.1. Potential for gene transfer[27]

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through HGT of DNA, or vertical gene flow via seed dispersal and cross-pollination. Considering the intended uses of cotton MON 531 × MON 1445, there are no requirements for scientific information on environmental safety assessment of accidental release or cultivation of cotton MON 531 × MON 1445. Therefore, vertical gene flow via seed dispersal and cross-pollination is not considered.

Plant to bacteria gene transfer

The recombinant DNA inserts in cotton MON 531 × MON 1445 could hypothetically be acquired through 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.

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 the 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 stabilised 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).

[27] Technical dossier / Section D6

Furthermore, the risk assessment of the 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 sources of exposure to the same genetic traits.

Hazard identification and characterisation

Cotton MON 531 × MON 1445 contains recombinant genes originating from bacteria, i.e., CP4 *epsps*, *aad*A, *npt*II, *ori*V, and the *nos* promoter (see Section 3.1.2 of the scientific opinion). It also contains a synthetic *cry1Ac* gene encoding for a Cry1Ac variant protein with 99.4 % amino acid sequence identity to a natural insecticidal Cry1Ac protein of a *B. thuringiensis* strain. The *cry1Ac* and the *npt*II genes are both under the control of 35S promoter originating from the *Cauliflower mosaic virus*. The CP4 *epsps* gene is under the control of the modified Figwort mosaic 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* and *cry1Ac* genes and their regulatory elements are taken up by bacteria, no selective advantage is anticipated, because EPSPS- and Cry-encoding genes already occur in various bacterial species in the environment. The expected widespread environmental presence of genetically diverse natural variants of EPSPS- and Cry-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 that it is highly unlikely that the CP4 *epsps* and *cry1Ac* genes transfer and establish in the genome of bacteria in the environment or in the gastrointestinal tract of humans or animals (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 531 × 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 531 × 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 to 10^{10} times compared with stabilisation by the process of illegitimate recombination encountered for constructs in which 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, as 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 contribute significantly to the HGT process.

Expression of the *npt*II gene under the control of the 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).

Hazard identification and characterisation indicates that HGT of the *npt*II gene cassette of cotton MON 531 × MON 1445 could lead to kanamycin- and neomycin-resistant bacteria emerging in some environments, especially in the gastrointestinal tract or faeces of humans or animals receiving diets containing DNA of MON 531 × MON 1445, 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 be substantially 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 derived from the 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.[28] 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.[29]

In the case of products containing recombinant 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 they may contain intact DNA. Exposure is also possible for products in which the transgenic DNA is more degraded but the DNA of gene length sizes could still be present. For instance, such DNA is expected to be present only in limited quantities in cottonseed meal due to effects of processing. No exposure is expected from highly processed and refined products, such as cottonseed oil and methylcellulose. In animal feeding, cotton products are used only in small amounts in the EU (FEDIOL, online), mainly due the presence of gossypol, which is highly toxic to non-ruminants (Verstraete, in press).[30] Even with accepted upper limits of 500 mg/kg gossypol in feed for ruminants[31], the feed source will contain only a low percentage of cottonseed meal. Because of the restricted dietary amounts, the effects of feed processing and degradation in the gastrointestinal tract and faeces the manure of animals fed with cotton MON 531 × MON 1445 will contain only very limited amounts of DNA of gene length size. Bacteria in soil or surface waters could be exposed to DNA from cotton MON 531 × MON 1445 in manure. Compared with usage as defined in the scope of this application, such exposure will be highly limited.

The probability of HGT depends on the presence of bacteria with the 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 optimised 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).

[28] Additional information, December 2010
[29] Additional information, December 2010
[30] 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
[31] 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

The 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 531 × 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.[32] DNA is substantially further degraded in the gastrointestinal tract of animals limiting the presence of gene-sized DNA fragments in this environment and in faeces (Jonas et al., 2001; Van den Eede et al., 2004). As cotton-derived products are only fed to animals in low amounts in the EU (FEDIOL, online; Verstraete, in press), the per animal exposure will be very low.

The *aad*A and *ori*V sequences that flank the *npt*II gene in cotton MON 531 × MON 1445 are present in naturally occurring bacteria in an arrangement that would allow double homologous recombination. The theoretical probability of horizontal transfer of the transgene sequences into bacteria is therefore higher compared with plant transgenes that do not have such flanking DNA sequences. The molecular characteristics of the inserted DNA in cotton MON 531 × MON 1445 facilitates homologous recombination with bacteria harbouring *aad*A and *ori*V sites in their DNA. Since such recombination sites are found 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, resistance genes other 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 that 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 531 × MON 1445.

Conclusion

The environmental risk assessment indicates no risk arising from a HGT of the *aad*A, CP4 *epsps* and *cry 1Ac* genes from cotton MON 531 × MON 1445 to bacteria because of a highly limited potential for transfer. However, it reveals that for products from cotton MON 531 × MON 1445 containing transgenic DNA, there is an increased likelihood of stabilisation of the *npt*II gene from plant DNA in bacteria compared with plants not including sites for double homologous recombination. This increased likelihood of transfer and the environmental effects must, however, be seen in the context of the occurrence and the horizontal mobility of resistance traits present in various bacterial communities, 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 DNA exposure per animal and hence the low frequency of gene transfer from MON 531 × MON 1445 to bacteria compared with gene transfer frequencies between bacteria, the GMO Panel concludes that material from cotton MON 531 × MON 1445 is highly unlikely to contribute to the environmental prevalence of *npt*II. In summary, the analysis of HGT from

[32] Additional information, December 2010

cotton MON 531 × 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.

6.1.2.2. Interactions of the GM plant with target organisms[33]

Due to the intended uses of cotton MON 531 × MON 1445, which exclude cultivation and import of viable plant parts, interactions of cotton MON 531 × MON 1445 with target organisms were not considered an issue by the EFSA GMO Panel.

6.1.2.3. Interactions of the GM plant with non-target organisms[34]

Due to the intended uses of cotton MON 531 × MON 1445, which exclude cultivation and import of viable plant parts, interactions of cotton MON 531 × MON 1445 with non-target organisms were not considered an issue by the EFSA GMO Panel.

6.1.2.4. Potential interactions with the abiotic environment and biogeochemical cycles[35]

Due to the intended uses of cotton MON 531 × MON 1445, which exclude cultivation and import of viable plant parts, interactions of cotton MON 531 × MON 1445 with the abiotic environment were not considered an issue by the EFSA GMO Panel.

6.1.3. Post-market environmental monitoring[36]

Considering the scope of the applications EFSA-GMO-UK-2005-09 and EFSA-GMO-RX-MON531×MON1445 for food and feed produced from cotton MON 531 × MON 1445, a post-market environmental monitoring plan for cotton MON 531 × MON 1445 is not required.

6.2. Conclusion

Cotton MON 531 × MON 1445 is being assessed for food and feed produced from cotton MON 531 × MON 1445; the scope includes only products produced from cotton MON 531 × MON 1445 that contain no viable plant parts. Therefore, there are no requirements for scientific information on the environmental risks associated with the accidental release or cultivation of cotton MON 531 × MON 1445. No risk arising from a HGT of the CP4 *epsps* and *cry1Ac* genes from cotton MON 531 × MON 1445 to bacteria has been identified. The hazard of an increased likelihood of stabilisation of the *npt*II gene from cotton MON 531 × MON 1445 in bacteria was postulated. However, considering the overall limited occurrence of horizontal transfer of DNA in plant material to bacteria, and the very low exposure to DNA from cotton MON 531 × MON 1445, the EFSA GMO Panel concludes that it is highly unlikely that cotton MON 531 × MON 1445 will contribute to the environmental prevalence of *npt*II genes. The analysis of HGT from cotton MON 531 × 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 531 × MON 1445 with non-target organisms and the abiotic environment were not considered to be an issue owing to the low level of exposure. A post-market environmental monitoring plan for cotton MON 531 × MON 1445 is not required.

OVERALL CONCLUSIONS

The EFSA GMO Panel was requested to carry out a scientific assessment of cotton MON 531 × MON 1445 in the context of two applications. Whereas application EFSA-GMO-UK-2005-09 is for the placing on the market of food and feed products derived from cotton MON 531 × MON 1445, the

[33] Technical dossier / Sections D8 and D9.4
[34] Technical dossier / Section D9.5
[35] Technical dossier / Sections D9.8 and D10
[36] Technical dossier / Section D11

scope of EFSA-GMO-RX-MON531×MON1445 covers the renewal of authorisation of (1) food additives produced from cotton MON 531 × MON 1445, and (2) feed produced from cotton MON 531 × 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.

In delivering its Scientific Opinion, the EFSA GMO Panel considered applications EFSA-GMO-UK-2005-09 and EFSA-GMO-RX-MON531×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. Further information from applications for the renewal of the authorisation for continued marketing of single cotton events MON 531 and MON 1445 was also taken into account. In accordance with its Guidance Document for renewal of authorisations of existing GMO products (EFSA, 2006b), the EFSA GMO Panel took into account the new information, experience and data on cotton MON 531 × MON 1445 that became available during the authorisation period.

The EFSA GMO Panel is of the opinion that the molecular characterisation data provided for cotton MON 531 × MON 1445 are sufficient to conclude on this part of the risk assessment. The integrity of the functional inserts in the MON 531 and MON 1445 events was retained in cotton MON 531 × MON 1445. Genetic and phenotypic stability of the inserts was demonstrated. The overall levels of the Cry1Ac and CP4 EPSPS proteins were comparable to those of the corresponding single cotton events MON 531 and MON 1445. The EFSA GMO Panel concludes that the molecular characterisation does not indicate safety issues.

Previous evaluations of the single cotton events MON 531 and MON 1445 showed that they do not differ compositionally, agronomically or phenotypically from their respective conventional counterparts, except for the introduced traits. The results of the comparative analysis of cotton MON 531 × MON 1445 and its conventional counterpart indicated that no biologically relevant differences were identified in the compositional, phenotypic or agronomic characteristics, and that the composition of cotton MON 531 × MON 1445 fell within the range of non-GM cotton varieties, except for the presence of the newly expressed Cry1Ac, CP4 EPSPS and NPTII proteins. Based on the assessment of the data available, the EFSA GMO Panel is of the opinion that crossing cotton MON 531 and cotton MON 1445 to produce cotton MON 531 × MON 1445 does not result in interactions that cause compositional, agronomic or phenotypic changes that would raise a safety issue.

The safety of the Cry1Ac, CP4 EPSPS and NPTII proteins expressed in MON 531 and MON 1445 was assessed previously, and no safety issues were identified for humans and animals. In addition, the EFSA GMO Panel considers that it is unlikely that the allergenicity of the whole cotton MON 531 × MON 1445 has been changed. A feeding study with catfish confirmed that the nutritional properties of cotton MON 531 × MON 1445 are not different from those of commercial non-GM cotton varieties. Potential interactions between the cotton events with respect to an effect on human and animal health were the focus of the assessment on food/feed issues. On the basis of the known functional characteristics and modes of action of the newly expressed proteins (Cry1Ac, CP4 EPSPS and NPTII), the EFSA GMO Panel considers it unlikely that interactions among these proteins would occur that would raise any safety issue. Thus, the Panel concludes that cotton MON 531 × MON 1445-derived products are as safe and as nutritious as products derived from its conventional counterpart and commercial cotton varieties in the context of their intended uses.

The scope of application EFSA-GMO-RX-MON531×MON1445 covers only food and feed products derived from cotton MON 531 × MON 1445 that contain no viable plant parts. Therefore, there are no requirements for scientific information on the environmental risks associated with the accidental release or cultivation of cotton MON 531 × MON 1445. No risk arising from a HGT of the CP4 *epsps* and *cry1Ac* genes from cotton MON 531 × 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 531 × 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 531 × MON 1445 in bacteria. However, considering the overall limited occurrence of horizontal transfer of DNA in plant material to bacteria, and the very low exposure to DNA from cotton MON 531 × MON 1445, the EFSA GMO Panel concludes that it is highly unlikely that cotton MON 531 × MON 1445 will contribute to the environmental prevalence of *npt*II genes. The assessment of HGT from cotton MON 531 × 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 531 × MON 1445 with non-target organisms and the abiotic environment were not considered an issue owing to the low level of exposure. A post-market environmental monitoring plan for cotton MON 531 × MON 1445 is not required.

In conclusion, the EFSA GMO Panel considers that information available for cotton MON 531 × MON 1445 addresses the questions raised by the Member States and that products derived from cotton MON 531 × MON 1445 are as safe as those from the conventional counterpart in the context of their intended uses.

DOCUMENTATION PROVIDED TO EFSA IN RELATION TO EFSA-GMO-UK-2005-09

1. Letter from the Competent Authority of the United Kingdom, received 6 January 2005, concerning a request for placing on the market of cotton MON 531 × MON 1445 in accordance with Regulation (EC) No 1829/2003.
2. Acknowledgement letter, dated 11 January 2005, from EFSA to the Competent Authority of the United Kingdom.
3. Letter from EFSA to applicant, dated 11 March 2005, requesting clarifications on the scope under completeness check.
4. Letter from applicant to EFSA, received 21 March 2005, providing clarifications on the scope under completeness check.
5. Letter from applicant to EFSA, received 23 March 2005, providing additional information under completeness check.
6. Letter from applicant to EFSA, dated 12 April 2005, providing complementary information under completeness check.
7. Letter from EFSA to applicant, dated 12 July 2005, delivering the 'Statement of Validity' for application EFSA-GMO-UK-2005-09, cotton MON 531 × MON 1445 submitted by Monsanto under Regulation (EC) No 1829/2003.
8. Letter from EFSA to applicant, dated 26 July 2005, requesting additional information and stopping the clock on request from the EURL-GMFF.
9. Letter from EFSA to applicant, dated 31 January 2006, requesting additional information and stopping the clock.
10. Fax from the EURL-GMFF to EFSA received on 22/05/2006 acknowledging the reception of additional information sent to EURL-GMFF by the applicant on 18/05/2006
11. Letter from EFSA to applicant, dated 30 June 2006, restarting the clock on request from the EURL_GMFF and maintaining it stopped for EFSA.
12. Letter from applicant to EFSA, received 16 August 2007, providing additional information.
13. Letter from applicant to EFSA, received 11 December 2007, providing additional information.

14. Letter from EFSA to applicant, dated 24 June 2008, requesting additional information and maintaining the clock stopped.
15. Letter from applicant to EFSA, received 4 July 2008, providing additional information.
16. Letter from EFSA to applicant, dated 9 September 2008, requesting additional information and maintaining the clock stopped.
17. Letter from EFSA to applicant, dated 12 September 2008, requesting additional information and maintaining the clock stopped.
18. Letter from applicant to EFSA, received 30 September 2008, providing additional information.
19. Letter from EFSA to applicant, dated 23 October 2008, requesting additional information and maintaining the clock stopped.
20. Letter from applicant to EFSA, received 14 November 2008, providing additional information.
21. Letter from applicant to EFSA, received 18 November 2008, providing additional information.
22. Letter from EFSA to applicant, dated 30 January 2009, requesting additional information and maintaining the clock stopped.
23. Letter from applicant to EFSA, received 2 March 2009, providing additional information.
24. Letter from EFSA to applicant, dated 7 April 2009, requesting additional information and maintaining the clock stopped.
25. Letter from applicant to EFSA, received 23 April 2009, providing additional information.
26. Letter from EFSA to applicant, dated 27 April 2009, requesting additional information and maintaining the clock stopped.
27. Letter from applicant to EFSA, received 23 June 2009, providing additional information.
28. Letter from EFSA to applicant, dated 24 July 2009, requesting additional information and maintaining the clock stopped.
29. Letter from EFSA to applicant, dated 18 September 2009, requesting additional information and maintaining the clock stopped.
30. Letter from applicant to EFSA, received 3 February 2010, providing additional information.
31. Letter from EFSA to applicant, dated 27 October 2009, requesting additional information and maintaining the clock stopped.
32. Letter from applicant to EFSA, received 9 December 2009, providing additional information.
33. Letter from EFSA to applicant, dated 4 February 2010, requesting additional information and maintaining the clock stopped.
34. Letter from applicant to EFSA, received 17 March 2010, providing additional information.
35. Letter from EFSA to applicant, dated 27 May 2010, requesting additional information and maintaining the clock stopped.
36. Letter from applicant to EFSA, received 3 June 2010, providing additional information.
37. Letter from applicant to EFSA, received 8 June 2010, providing additional information.
38. Letter from EFSA to applicant, dated 2 August 2010, requesting clarifications.
39. Letter from applicant to EFSA, received 14 September 2010, providing additional information.
40. Letter from applicant to EFSA, 15 September 2010, providing clarifications.
41. Letter from EFSA to applicant, dated 15 December 2011 maintaining the clock stopped.
42. Letter from EFSA to applicant, dated 16 December 2011, restarting the clock.

DOCUMENTATION PROVIDED TO EFSA IN RELATION TO EFSA-GMO-RX-MON 531 × MON 1445

1. Letter from the European Commission, received 29 June 2007, concerning a request for the renewal of the authorisation of the placing on the market of cotton MON 531 × MON 1445 in accordance with Regulation (EC) No 1829/2003.
2. Acknowledgement letter, dated 20 July 2007, from EFSA to the European Commission.
3. Letter from EFSA to applicant, dated 21 January 2008, requesting additional information under completeness check.
4. Letter from applicant to EFSA, received 26 February 2008, providing additional information under completeness check.
5. Letter from EFSA to applicant, dated 12 March 2008, delivering the 'Statement of Validity' for application EFSA-GMO-RX-MON531×MON1445, cotton MON 531 × MON 1445 submitted for renewal under Regulation (EC) No 1829/2003 by Monsanto.
6. Letter from EFSA to applicant, dated 13 March 2008, requesting additional information and stopping the clock.
7. Letter from EFSA to applicant, dated 24 June 2008, requesting additional information and maintaining the clock stopped.
8. Letter from applicant to EFSA, received 4 July 2008, providing additional information.
9. Letter from EFSA to applicant, dated 9 September 2008, requesting additional information and maintaining the clock stopped.
10. Letter from EFSA to applicant, dated 12 September 2008, requesting additional information and maintaining the clock stopped.
11. Letter from applicant to EFSA, received 30 September 2008, providing additional information.
12. Letter from EFSA to applicant, dated 23 October 2008, requesting additional information and maintaining the clock stopped.
13. Letter from applicant to EFSA, received 14 November 2008, providing additional information.
14. Letter from applicant to EFSA, received 18 November 2008, providing additional information.
15. Letter from EFSA to applicant, dated 30 January 2009, requesting additional information and maintaining the clock stopped.
16. Letter from applicant to EFSA, received 2 March 2009, providing additional information.
17. Letter from EFSA to applicant, dated 7 April 2009, requesting additional information and maintaining the clock stopped.
18. Letter from applicant to EFSA, received 23 April 2009, providing additional information.
19. Letter from EFSA to applicant, dated 28 April 2009, requesting additional information and maintaining the clock stopped.
20. Letter from applicant to EFSA, received 23 June 2009, providing additional information.
21. Letter from EFSA to applicant, dated 24 July 2009, requesting additional information and maintaining the clock stopped.
22. Letter from EFSA to applicant, dated 18 September 2009, requesting additional information and maintaining the clock stopped.
23. Letter from applicant to EFSA, received 3 February 2010, providing additional information.

24. Letter from EFSA to applicant, dated 27 October 2009, requesting additional information and maintaining the clock stopped.
25. Letter from applicant to EFSA, received 9 December 2009, providing additional information.
26. Letter from EFSA to applicant, dated 4 February 2010, requesting additional information and maintaining the clock stopped.
27. Letter from applicant to EFSA, received 17 March 2010, providing additional information.
28. Letter from EFSA to applicant, dated 27 May 2010, requesting additional information and maintaining the clock stopped.
29. Letter from applicant to EFSA, received 3 June 2010, providing additional information.
30. Letter from applicant to EFSA, received 8 June 2010, providing additional information.
31. Letter from EFSA to applicant, dated 2 August 2010, requesting clarifications.
32. Letter from applicant to EFSA, received 14 September 2010, providing additional information.
33. Letter from applicant to EFSA, 15 September 2010, providing clarifications.
34. Letter from EFSA to applicant, dated 15 December 2011 maintaining the clock stopped.
35. Letter from EFSA to applicant, dated 16 December 2011, restarting the clock

REFERENCES

Assaad FF and Signer ER, 1990. *Cauliflower mosaic virus* P35S promoter activity in *Echerichia coli*. Molecular Genetics and Genomics, 223, 517-520.

Atkins FM, Wilson M and Bock SA, 1988. Cottonseed hypersensitivity: new concerns over an old problem. Journal of Allergy and Clinical Immunology, 82, 242-250.

Brigulla M and Wackernagel W, 2010. Molecular aspects of gene transfer and foreign DNA acquisition in prokaryotes with regard to safety issues. Applied Microbiology and Biotechnology, 86, 1027-1041.

Codex Alimentarius, 2009. Foods derived from modern biotechnology. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme. Rome, Italy, 85 pp.

De Vries J and Wackernagel W, 2002. Integration of foreign DNA during natural transformation of Acinetobacter sp. by homology-facilitated illegitimate recombination. Proceedings of the National Academy of Sciences USA, 99, 2094-2099.

Duggan PS, Chambers, PA, Heritage, J, and Forbes, JM, 2000. Survival of free DNA encoding antibiotic resistance from transgenic maize and the transformation activity of DNA in ovine saliva, ovine rumen fluid and silage effluent. FEMS Microbiology Letters, 191, 71-77.

Duggan PS, Chambers, PA, Heritage, J, and Forbes, JM, 2003. Fate of genetically modified maize DNA in the oral cavity and rumen of sheep. British Journal of Nutrition, 89, 159-166.

EFSA (European Food Safety Authority), 2006a. Guidance document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed. The EFSA Journal 99, 1-100.

EFSA (European Food Safety Authority), 2006b. Guidance document for the 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 Journal, 435, 1-4.

EFSA (European Food Safety Authority), 2007. Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events The EFSA Journal 512, 1-5.

EFSA (European Food Safety Authority), 2009. Consolidated presentation of the joint Scientific Opinion of the GMO and BIOHAZ Panels on the "Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants" and the Scientific Opinion of the GMO Panel on "Consequences of the Opinion on the Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants on Previous EFSA Assessments of Individual GM Plants. The EFSA Journal (2009) 1108, 1-8.

EFSA Panel on Genetically Modified Organisms (GMO), 2010. Scientific Opinion of the Panel on Genetically Modified Organisms (GMO) on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal, 8(7), 1700.

EFSA Panel on Genetically Modified Organisms (GMO), 2011a. Scientific Opinion on application EFSA-GMO-RX-MON531 for renewal of the authorisation for continued marketing of existing cottonseed oil, food additives, feed materials and feed additives produced from MON 531 cotton that were notified under Articles 8(1)(a), 8(1)(b) and 20(1)(b) of Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal, 9(9), 2373.

EFSA Panel on Genetically Modified Organisms (GMO), 2011b. 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. EFSA Journal, 9(12), 2479.

FEDIOL (Federation of EU Oil and Proteinmeal Industry), online. Statistics. Available from: http://www.fediol.be/site/index.php?section=11&menu=102

Genbank, 2010, online. Availaible from: http://www.ncbi.nlm.nih.gov/genbank/

Hülter N and Wackernagel, W, 2008. Double illegitimate recombination events integrate DNA segments through two different mechanisms during natural transformation of *Acinetobacter baylyi*. Molecular Microbiology, 67, 984-995.

Jonas DA, Elmadfa I, Engel KH, Heller KJ, Kozianowski G, König A, Müller D, Narbonne JF, Wackernagel W and Kleiner J, 2001. Safety considerations of DNA in food. Annals of Nutrition and Metabolism 45, 235-254.

Lewin A, Jacob D, Freytag B and Appel B, 1998. Gene expression in bacteria directed by plant-specific regulatory sequences. Transgenic Research, 7, 403-411.

Malanin G and Kalimo K, 1988. Angioedema and urticaria caused by cottonseed protein in wholegrain bread. Journal of Allergy and Clinical Immunology, 82, 261-264.

Mercer DK, Melville CM, Scott KP and Flint HJ, 1999a. Natural genetic transformation in the rumen bacterium *Streptococcus bovis* JB1. FEMS Microbiology Letters, 179, 485-490.

Mercer DK, Scott KP, Bruce-Johnson WA, Glover LA and Flint HJ, 1999b. Fate of free DNA and transformation of the oral bacterium *Streptococcus gordonii* DL1 by plasmid DNA in human saliva. Applied and Environmental Microbiology, 65, 6-10.

Mercer DK, Scott KP, Melville CM, Glover LA and Flint HJ, 2001. Transformation of an oral bacterium via chromosomal integration of free DNA in the presence of human saliva. FEMS Microbiology Letters, 200, 163-167.

Nordgård L, Nguyen T, Modtvelt T, Benno Y, Traavik T and Nielsen KM, 2007. Lack of detectable update of DNA by bacterial gut isolates grown in vitro and by *Acinetobacter baylyi* colonizing rodents in situ. Environmental Biosafety Research, 6, 149-160.

OECD (Organisation for Economic Co-operation and Development), 2009. Consensus document on compositional considerations for new varieties of cotton (*Gossypium hirsutum* and *Gossypium barbadense*): key food and feed nutrients and anti-nutrients. Series on the Safety of Novel Foods and Feeds, No 11. ENV/JM/MONO(2004)16.

Rizzi A, Raddadi N, Sorlini C, Nordgård L, Nielsen KM and Daffonchio D, 2012. The stability and degradation of dietary DNA in the gastrointestinal tract of mammals - implications for horizontal gene transfer and the biosafety of GMOs. Critical Reviews in Food Science and Nutrition, 52, 142-161.

van den Eede G, Aarts H, Bukh HJ, Corthier G, Flint HJ, Hammes W, Jacobsen B, Midtvedt T, van der Vossen J, von Wright A, Wackernagel W and Wilcks A, 2004. The relevance of gene transfer to the safety of food and feed derived from genetically modified (GM) plants. Food and Chemical Toxicology 42, 1127-1156.

Verstraete F, in press. Risk management of undesirable substances in feed following updated risk assessments. Toxicology and Applied Pharmacology, DOI:10.1016/j.taap.2010.09.015