

SCIENTIFIC OPINION

Scientific Opinion on applications (EFSA-GMO-UK-2008-57 and EFSA-GMO-RX-MON15985) for the placing on the market of insect-resistant genetically modified cotton MON 15985 for food and feed uses, import and processing¹, and for the renewal of authorisation of existing products produced from cotton MON 15985², both under Regulation (EC) No 1829/2003 from Monsanto

EFSA Panel on Genetically Modified Organisms (GMO)^{3,4}

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ABSTRACT

Cotton MON 15985 was developed by biolistic transformation of cotton MON 531 to express Cry2Ab2 and GUS in addition to the Cry1Ac and NPTII proteins. Cry proteins in MON 15985 confer resistance to major lepidopteran cotton pests, whereas the GUS and NPTII proteins were used as markers during product development. Molecular characterisation of MON 15985 did not give rise to safety issues. The EFSA GMO Panel could not conclude on the potential occurrence of unintended effects for agronomic and phenotypic characteristics owing to data limitations. Compositional data gave no indication of unintended effects for which further assessment was needed. The Panel concludes that cotton MON 15985, as described in these applications, is as safe and nutritious as its conventional counterpart and other non-genetically modified varieties, and considers it unlikely that the overall allergenicity of the whole plant is changed. Environmental risk assessment was restricted to the exposure through faecal material from animals fed with cotton products of MON 15985 and its accidental spillage. Following a weight of evidence approach and considering the poor ability of cotton to survive outside cultivated land, despite the agronomic and phenotypic data limitations, the Panel concludes that there is very low likelihood of any adverse environmental impacts. The *aadA* and *oriV* sequences in MON 15985 may facilitate the stabilisation of *nptII* through double homologous recombination. However, considering the limited presence of intact DNA from MON 15985 in feed and the limited occurrence of horizontal transfer of DNA from plant material to bacteria, the Panel concludes that it is highly unlikely that *nptII* from MON 15985 will be transferred to bacteria.

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¹ On request from the Competent Authority of the United Kingdom for an application (EFSA-GMO-UK-2008-57) submitted by Monsanto, Question No EFSA-Q-2008-385, adopted on 2 July 2014.

² On request from the European Commission for an application (EFSA-GMO-RX-MON15985) submitted by Monsanto, Question No EFSA-Q-2007-145, adopted on 2 July 2014.

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KEY WORDS

GMO, cotton, risk assessment, MON 15985, Genuity® Bollgard II®, insect resistance, Cry1Ac, Cry2Ab2

SUMMARY

Following requests 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-2008-57 and EFSA-GMO-RX-MON15985 respectively, both submitted by Monsanto under Regulation (EC) No 1829/2003⁵. While application EFSA-GMO-UK-2008-57 is for the placing on the market of cotton MON 15985 for food and feed uses, EFSA-GMO-RX-MON15985 is for the renewal of authorisation for continued marketing of:

- food additives produced from cotton MON 15985, authorised under Directive 89/107/EEC⁶;
- feed produced from cotton MON 15985 (feed materials and feed additives), authorised under Directive 70/524/EEC⁷.

After the date of entry into force of Regulation (EC) No 1829/2003, the products mentioned above were notified to the EC in accordance with Articles 8(1)(b) or 20(1)(b) of this Regulation and subsequently included in the European Union (EU) Register of authorised GMOs⁸.

Since both EFSA-GMO-UK-2008-57 and EFSA-GMO-RX-MON15985 cover cotton MON 15985, the EFSA GMO Panel provides a single scientific opinion, valid for both applications.

The EFSA GMO Panel evaluated cotton MON 15985 with reference to the scope and appropriate principles described in its guidance documents for the risk assessment of genetically modified (GM) plants and derived food and feed (EFSA, 2006a; EFSA GMO Panel, 2011a), environmental risk assessment (ERA) (EFSA GMO Panel, 2010a) and for renewal of authorisations of existing GMO products lawfully placed on the market (EFSA, 2006b). The scientific evaluation of the risk assessment included molecular characterisation of the inserted DNA and analysis of the expression of the corresponding proteins. An evaluation of the comparative analyses of compositional, agronomic and phenotypic characteristics was undertaken, and the safety of the newly expressed proteins and the whole food/feed was evaluated with respect to potential toxicity, allergenicity and nutritional wholesomeness. An evaluation of environmental impacts and the post-market environmental monitoring (PMEM) plan was also undertaken.

The scope of applications EFSA-GMO-UK-2008-57 and EFSA-GMO-RX-MON15985 covers the MON 15985 event in cotton species *Gossypium hirsutum* L. and *G. barbadense* L. The genus *Gossypium* consists of more than 50 species, two of which are the most commonly cultivated species (*G. hirsutum* and *G. barbadense*). The composition of cottonseed from *G. barbadense* does not differ from that of seed from *G. hirsutum* to the extent that a food and feed risk assessment of one species would not be applicable also to the other.

Cotton MON 15985 was obtained by the transformation of GM cotton MON 531 (unique identifier MON-ØØ531-6) with a DNA fragment carrying two expression cassettes: *cry2Ab2* and *uidA*. While expression of the Cry2Ab2 protein confers resistance to the major lepidopteran cotton pests including the cotton bollworm, tobacco budworm and the pink bollworm, the GUS E377K protein, produced by the *uidA* gene, was used as a histochemical marker during product development.

Cotton MON 531 has been developed to produce a synthetic variant of the Cry1Ac protein. In addition, cotton MON 531 contains a kanamycin resistance gene (*nptII*) under plant expression signals and the streptomycin/spectinomycin resistance gene *aadA* under the control of its bacterial promoter.

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, p. 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, p. 27–33.

⁷ Council Directive 70/524/EEC of 23 November 1970 concerning additives in feeding-stuffs. OJ L 270, p. 1–17.

⁸ http://ec.europa.eu/food/dyna/gm_register/index_en.cfm

Cotton MON 531 has been assessed previously (EFSA GMO Panel, 2011b) on the basis of experimental data. No concerns were identified for human and animal health and the environment. The molecular characterisation data provided for cotton MON 15985 did not give rise safety issues.

The EFSA GMO Panel could not complete the assessment of the agronomic and phenotypic characteristics of cotton MON 15985 on the basis of the data provided (a single season and fewer than eight sites (EFSA, 2006a; EFSA GMO Panel 2011a)). Therefore, the EFSA GMO Panel could not conclude on the potential occurrence of unintended effects based on the outcome of the agronomic and phenotypic analysis. The EFSA GMO Panel concludes that the compositional data give no indication that the genetic modification induces unintended effects for which further assessment is needed. The EFSA GMO Panel concludes that cotton MON 15985 is as safe and nutritious as its conventional counterpart and that it is unlikely that the overall allergenicity of the whole plant is changed.

Applications EFSA-GMO-UK-2008-57 and EFSA-GMO-RX-MON15985 cover the import, processing, and food and feed uses of cotton MON 15985. Therefore, there is no requirement for scientific information on possible environmental effects associated with the cultivation of cotton MON 15985. In accordance with its guidance document on the ERA of GM plants (EFSA, 2010a), the EFSA GMO Panel follows a weight of evidence approach in collating and assessing appropriate information from various data sources (e.g. molecular and compositional data, available agronomic and phenotypic data from field trials performed by the applicant, literature) in order to assess the likelihood of unintended effects on the environment. Notwithstanding the incompleteness of the agronomic and phenotypic dataset, the EFSA GMO Panel followed a weight of evidence approach and, considering the scope of this application and the poor ability of cotton to survive outside cultivated fields, concluded that there is very low likelihood of any adverse environmental impacts due to the accidental release into the environment of viable seeds from cotton MON 15985. The *aadA* and *oriV* sequences in MON 15985 may facilitate the stabilisation of *nptII* through double homologous recombination in plasmid sequences in the environment. However, considering the limited presence of intact DNA from MON 15985 in feed and processed feed owing to the low percentage of cotton plant material allowed in feed products, and the limited occurrence of horizontal transfer of DNA from plant material to bacteria, the EFSA GMO Panel concludes that it is highly unlikely that cotton MON 15985 will contribute to the environmental prevalence of *nptII* genes. The scope of the PMEM plan provided by the applicant is in line with the intended uses of cotton MON 15985. Furthermore, the EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

In delivering its scientific opinion, the EFSA GMO Panel considered applications EFSA-GMO-UK-2008-57 and EFSA-GMO-RX-MON15985, additional information submitted by the applicant on request of the Panel, the scientific comments submitted by Member States and relevant scientific publications. In accordance with its guidance document for renewal of authorisations of existing GMO products (EFSA, 2006b), the EFSA GMO Panel took into account the new information, experience and data on cotton MON 15985 that became available during the authorisation period.

The EFSA GMO Panel considers that the dossiers presented by the applicant had deficiency in the data set relative to agronomic and phenotypic trials, however the EFSA GMO Panel concludes that cotton MON 15985, as described in applications EFSA-GMO-UK-2008-57 and EFSA-GMO-RX-MON15985, is as safe as its conventional counterpart and non-GM cotton commercial varieties, and is unlikely to have adverse effects on human and animal health and the environment in the context of the scope of these applications.

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BACKGROUND

On 22 May 2008, the European Food Safety Authority (EFSA) received from the United Kingdom Competent Authority an application (EFSA-GMO-UK-2008-57) for authorisation of genetically modified (GM) cotton MON 15985 (Unique Identifier MON-15985-7) submitted by Monsanto within the framework of Regulation (EC) No 1829/2003 on GM food and feed. After receiving the application EFSA-GMO-UK-2008-57, 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 website⁹. 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 20 August 2008, 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-MON15985) submitted under Regulation (EC) No 1829/2003 for renewal of the authorisation of food additives and feed produced from cotton MON 15985 (feed materials and feed additives).

The scope of the renewal application, as described in the EU Register of authorised GMOs¹⁰, covers the continued marketing of:

- food additives produced from cotton MON 15985, authorised under Directive 89/107/EEC;
- feed produced from cotton MON 15985 (feed materials and feed additives), authorised under Directive 70/524/EEC.

After receiving the renewal application EFSA-GMO-RX-MON15985 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 website¹¹. 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 18 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-2008-57 and EFSA-GMO-RX-MON15985 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/EC¹², to request their scientific opinion. The Member State bodies had 3 months after the date of receipt of the valid application (until 20 November 2008 and 18 June 2008, respectively) within which to make their opinion known.

The scope of applications EFSA-GMO-UK-2008-57 and EFSA-GMO-RX-MON15985 covers the MON 15985 event in cotton species *Gossypium hirsutum* L. and *G. barbadense* L.¹³.

The EFSA GMO Panel carried out an evaluation of the risk assessment of the applications on cotton MON 15985 in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. The Panel took into account the appropriate principles described in its guidance documents for the risk assessment of GM plants and derived food and feed (EFSA, 2006a; EFSA GMO Panel 2011a), environmental risk assessment (EFSA GMO Panel, 2010a) and for renewal of authorisations of existing GMO products lawfully placed on the market (EFSA, 2006b). Furthermore, the scientific

⁹ <http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2008-385>

¹⁰ http://ec.europa.eu/food/dyna/gm_register/gm_register_auth.cfm?pr_id=6

¹¹ <http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2007-145>

¹² 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 L 106, p. 1–39.

¹³ Clarification from the applicant: 15/09/2010.

comments of Member States, the additional information provided by the applicant and relevant scientific publications were also taken into consideration.

For EFSA-GMO-UK-2008-57, the EFSA GMO Panel requested additional information from the applicant on 20 August 2008, 24 November 2008, 7 April 2009, 28 May 2009, 19 September 2009, 15 March 2010, 4 October 2010, 31 January 2011, 5 December 2011, 6 July 2012, 9 January 2012, 3 June 2013, and on 23 August 2013. The applicant provided additional information on 15 September 2010, 2 December 2010, 11 April 2011, 14 September 2012, 5 November 2012, 12 March 2013, 5 November 2013, and on 11 November 2013.

For EFSA-GMO-RX-MON15985, the EFSA GMO Panel requested additional information from the applicant on 26 May 2008, 24 November 2008, 7 April 2009, 26 May 2009, 18 September 2009, 12 March 2010, 4 October 2010, 31 January 2011, 5 December 2011, 6 July 2012, 9 January 2013, 3 June 2013, and on 23 August 2013. The applicant provided additional information on 28 October 2008, 9 March 2009, 19 May 2009, 18 January 2010, 30 June 2010, 15 September 2010, 29 September 2010, 2 December 2010, 11 April 2011, 14 September 2012, 5 November 2012, 12 March 2013, 5 November 2013, and on 11 November 2013.

In giving its scientific opinion on cotton MON 15985 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 15985 (Unique Identifier: MON-15985-7) in the context of applications EFSA-GMO-UK-2008-57 and EFSA-GMO-RX-MON15985. While application EFSA-GMO-UK-2008-57 is for the placing on the market cotton MON 15985 for food and feed uses, the scope of EFSA-GMO-RX-MON15985 covers the renewal of authorisation of (1) food additives produced from cotton MON 15985, authorised under Directive 89/107/EEC; (2) feed produced from cotton MON 15985 (feed materials and feed additives), authorised under Directive 70/524/EEC notified to the EC according to Articles 8(1)(b) or 20(1)(b) of this Regulation (EC) No 1829/2003, respectively.

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

Cotton MON 15985 (Unique Identifier MON-15985-7) is assessed with reference to its intended uses, taking account of the appropriate principles described in the guidance documents of the EFSA Panel on Genetically Modified Organisms (EFSA GMO Panel) for the risk assessment of genetically modified (GM) plants and derived food and feed (EFSA, 2006a; EFSA GMO Panel, 2011a), environmental risk assessment (ERA) (EFSA GMO Panel, 2010a) and for the renewal of authorisations of existing GMO products lawfully placed on the market (EFSA, 2006b). The risk assessment presented here is based on the information provided in the applications relating to cotton MON 15985, additional information from the applicant, scientific comments raised by Member States and relevant scientific publications.

The scope of applications EFSA-GMO-UK-2008-57 and EFSA-GMO-RX-MON15985 is for food and feed uses, for food additives and feed produced from cotton MON 15985 and for import and processing; it does not include cultivation in the EU. Thus, cotton MON 15985 will be imported into the EU for the above-listed uses in the same way as any commercial cotton variety.

To obtain cotton MON 15985, *Gossypium hirsutum* L. was genetically transformed; however, the scope of applications EFSA-GMO-UK-2008-57 and EFSA-GMO-RX-MON15985 covers the MON 15985 event in cotton species *G. hirsutum* L. and *G. barbadense* L.¹⁴. Since there are no known genetic barriers to interspecies hybridisation between the tetraploid *Gossypium* species (Percival et al., 1999), the MON 15985 event could possibly be introgressed in *G. barbadense* through conventional breeding. At the request of the EFSA GMO Panel, the applicant provided information that the composition of cottonseed from *G. barbadense* does not differ from that of *G. hirsutum* regarding nutrients, anti-nutrients and toxicants, to such an extent that a food and feed risk assessment of one of these species would not also be applicable for the other species¹⁵. Therefore, the food and feed risk assessment of the MON 15985 event in cotton considered in this opinion is applicable to both *G. barbadense* and *G. hirsutum*.

Cotton MON 15985 was obtained by the transformation of GM cotton MON 531 (Unique Identifier MON-ØØ531-6) with a DNA fragment carrying two expression cassettes: *cry2Ab2* and *uidA*. Expression of the Cry2Ab2 protein confers resistance to major lepidopteran cotton pests including the cotton bollworm, tobacco budworm and the pink bollworm, while the GUS E377K protein, produced by the *uidA* gene, was used as a histochemical marker during product development.

Cotton MON 531 has been developed to produce a synthetic variant of the Cry1Ac protein. In addition, cotton MON 531 contains a kanamycin resistance gene (*nptII*) under plant expression signals and the streptomycin/spectinomycin resistance gene *aadA* under the control of its bacterial promoter. Cotton MON 531 has been assessed previously (EFSA GMO Panel, 2011b) on the basis of experimental data. No concerns for human and animal health and the environment were identified.

The genetic modifications in cotton MON 15985 are intended to improve agronomic performance only and are not intended to influence the nutritional properties, processing characteristics or overall use of cotton as a crop.

Cotton MON 15985 was first commercially grown in 2003 in the USA and in Australia, and later as the combined-trait product MON 15985 × MON 1445. In 2006, cottons containing event MON 15985 amounted to 7 % and 97 % of total cotton production in the USA and Australia, respectively. Most of this was cotton MON 15985 × MON 1445¹⁶.

¹⁴ Clarification from the applicant: 15/09/2010.

¹⁵ Additional information: 11/04/2011.

¹⁶ EFSA applications EFSA-GMO-UK-2008-58 and EFSA-GMO-RX-MON15985xMON1445.

Based on import data of cottonseed meal from cotton MON 15985-producing countries into the countries of the European Union (EU)¹⁷, the applicant has estimated that around 0.035 % of cottonseed meal used in the EU might be derived from cotton MON 15985 and its combined-trait products. It should be noted, however, that the calculation yielding these figures is based on several assumptions and may vary between Member States.

2. Issues raised by the Member States

The comments raised by Member States are addressed in Annex G of the relevant EFSA overall opinion and were taken into consideration during the evaluation of the risk assessment^{18,19}.

3. Molecular characterisation

3.1. Evaluation of relevant scientific data

Cotton MON 15985 was obtained by the transformation of GM cotton MON 531, previously assessed by EFSA (EFSA GMO Panel, 2011b). Therefore, molecular characterisation of cotton MON 15985 includes both a summary of event MON 531 and the description of the second genetic modification, leading to cotton MON 15985.

3.1.1. Summary of the previous evaluation of event MON 531, including newly provided information

Cotton MON 531 contains two insertions, one functional and the other non-functional. The functional insert contains 7 916 bp of the transforming PV-GHBK04 plasmid, extending from the right transfer-DNA (T-DNA) border (RB) through the *cry1Ac* expression cassette, the *aadA* gene, the *nptII* expression cassette up to the *oriV* genetic element. In addition, another 3' portion of the *cry1Ac* expression cassette up to the RB is linked to the complete *cry1Ac* expression cassette in opposite orientation, arranged as an inverted repeat. The non-functional insert of 242 bp consists of the RB and a portion of the 7S 3' transcriptional termination sequence. Molecular characterisation of cotton MON 531 has been described and assessed previously by the EFSA GMO Panel (EFSA GMO Panel, 2011b). Cotton MON 531 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.

Updated bioinformatic analyses²⁰ of the insertion sites indicated that the functional insert did not disrupt known endogenous genes. Flanking sequences of the non-functional insert suggest that the insertion occurred in a 26S ribosomal RNA (rRNA) gene. Since rRNA genes are present in several copies in the genome (Ide et al., 2010), disruption of a single copy is unlikely to have an effect on the characteristics of the plant.

In order to assess whether the open reading frames (ORFs) present within the inserts and spanning the junction sites give rise to any safety issues, their putative translation products were compared for similarities to known allergens and toxins by using suitable algorithms and appropriate databases²¹. None of the ORF-derived amino acid sequences identified at the junctions and in the inserted sequences showed significant similarities with known toxins. Allergen search identified a 10-amino acid-long stretch at the 5' end of the 7S transcriptional terminators, showing identity to beta-conglycinin- α storage protein (alternative name of the 7S seed storage protein, of which the coding gene is the source of the 7S terminator). These 10 amino acid residues correspond to the carboxyl-terminus of the 7S seed storage protein. Since 36 nt upstream of the corresponding DNA

¹⁷ FAO Statistics data of import of cottonseed meal in countries of the European Union over the years 2003 to 2005. <http://faostat.fao.org>

¹⁸ <http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2008-385>

¹⁹ <http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2007-145>

²⁰ Additional information: 05/11/2013.

²¹ Additional information: 05/11/2013.

fragment there is a stop codon in the same reading frame, and no start codon is present in between, the translation of this sequence is highly unlikely.

Review of the scientific literature covering the period since the publication of the last EFSA scientific opinion on cotton MON 531 (EFSA GMO Panel, 2011b) identified no molecular characterisation-related hazards.

Consequently, the EFSA GMO Panel considers that its previous conclusions on the safety of cotton MON 531 event remain valid.

3.1.2. Transformation process and vector constructs

Cotton MON 15985 was developed by particle bombardment of cotton MON 531 meristems²². The DNA used in the transformation was a 6 091 bp linear *KpnI* fragment derived from plasmid PV-GHBK11. The DNA fragment contained two adjacent expression cassettes. One expression cassette contained the *Escherichia coli uidA* coding sequence under the control of the e35S promoter and the 3' termination signals of the *Agrobacterium tumefaciens* nopaline synthase (*nos*) gene. The *uidA* gene encodes β -D-glucuronidase, which catalyses the hydrolysis of a range of β -D-glucuronides, including the chromogenic artificial substrate p-nitrophenyl- β -D-glucuronide. It was used as a histochemical marker (reporter) for transgenic tissues. No selectable markers were used. The second expression cassette contained the e35S promoter, the 5' untranslated leader sequence of the *Petunia* heat shock protein 70, the N-terminal chloroplast transit peptide from the *Arabidopsis thaliana epsps* gene, the coding sequence of a synthetic *cry2Ab2* gene and the 3' termination signals of the *A. tumefaciens nos* gene. The resulting Cry2Ab2 protein differs from that of the native Cry2Ab protein from *Bacillus thuringiensis* by five amino acids at the N-terminus, which corresponds to the predicted region of the chloroplast transit peptide remaining after processing and a residue introduced for cloning purposes²³.

This second genetic modification is referred to as MON 15947. Genetically fixed germplasm, homozygous for both *cry1Ac* and *cry2Ab2* (from MON 531 and MON 15947, respectively) was produced by traditional breeding processes including stabilisation, backcrossing and selfing, and is referred to as MON 15985²⁴.

3.1.3. Transgene constructs in the GM plant

Molecular characterisation of cotton MON 15985 was performed by Southern analysis, polymerase chain reaction (PCR) and DNA sequence analysis, in order to determine insert copy number, size and organisation of the inserted sequences and to confirm the absence of plasmid backbone sequences²⁵. The approach used was acceptable in terms of both coverage and sensitivity.

Southern analysis of cotton control DNA, cotton MON 531 and cotton MON 15985 DNA digested separately with two different restriction enzymes, one cutting inside the expected insert sequence and one not cutting, using the PV-GHBK11 plasmid as a probe indicated the integration of a single MON 15947 insert into the cotton genome. This was supported by PCR analysis of five overlapping regions that span the entire length of the insert and by sequence analysis. The integrity of the functional insert of event MON 531 in the R₃ generation of MON 15985 has been demonstrated by Southern analysis spanning the flanking regions²⁶. Therefore, there is no indication of rearrangements resulting from an interaction between the events. The absence of additional DNA sequences derived from the vector PV-GHBK11 in MON 15985 plants has been confirmed by Southern analysis using probes that cover the entire sequence of the vector backbone.

²² Technical dossier, Section C1.

²³ Technical dossier, Sections C2 and C3.

²⁴ Technical dossier, Section A6.

²⁵ Technical dossier, Section D2.

²⁶ Additional information: 19/05/2009.

The nucleotide sequence of the MON 15947 insert in cotton MON 15985 has been determined in its entirety. The insert contains 5719 bp derived from the *KpnI* fragment of PV-GHBK11 plasmid used for transformation. At the 5' end 307 bp and at the 3' end 66 bp of the *KpnI* fragment are missing from the transformed plant. The deduced amino acid sequence of the coding sequence of *cry2Ab2* is as expected from the PV-GHBK11 sequence, but the inserted β -D-glucuronidase sequence differs by one amino acid (E377K). Flanking sequences extending 1599 bp from the 5' end and 636 bp from the 3' end of the MON 15947 insert were also determined²⁷.

Updated bioinformatic analyses²⁸ of the insertion site indicated that the MON 15947 insert did not disrupt known endogenous genes. During the transformation process, 1 847 bp of additional DNA was co-inserted with the intended sequences. The 5' flank of the MON 15947 insert consists of 1 524 bp of additional DNA, of which 389 bp shows similarity to chloroplast DNA and 124 bp to *A. thaliana* putative dynamin-like protein cDNA and 1 011 bp represents unidentified DNA. The 3' flanking sequence consist of 323 bp unidentified DNA. The chloroplast DNA inserted at the 5' flank is homologous to a part of NADH dehydrogenase subunit B that does not give rise to any safety issues.

In order to assess whether the ORFs present within the inserts (including the DNA co-inserted with the MON 15947 insert) and spanning the junction sites give rise to any safety issues, their putative translation products were compared for similarities to known allergens and toxins by using suitable algorithms and appropriate databases²⁹. None of the ORF-derived amino acid sequences identified at the junctions and in the inserted sequences showed significant similarities with known toxins or allergens. These 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 give rise to a safety issue.

3.1.4. Information on the expression of the insert

Cotton MON 15985 contains two inserts: (1) the MON 531 insert with the *cry1Ac*, *nptII* and *aadA* genes and (2) the MON 15947 insert with the *cry2Ab2* and *uidA* genes. The expression levels of the Cry1Ac, NPTII, Cry2Ab2 and β -D-glucuronidase proteins were measured by enzyme-linked immunosorbent assay (ELISA) in different samples of cotton MON 15985 cultivated in two field trials in the USA in 1998 (eight locations) and in 2001 (five locations). All locations represented major cotton-growing regions of the USA³⁰. The mean values and ranges of the protein levels in the seeds are summarised in Table 1. The expression levels of the Cry1Ac and NPTII proteins were similar between MON 15985 and MON 531 when compared within the same year and location. The expression levels of Cry2Ab2 and β -D-glucuronidase were similar in MON 15985 and MON 15947 in the 2001 trial. As expected, AAD protein was not detected in any of the samples analysed since the *aadA* gene is under the control of a prokaryotic promoter. Substantial changes in protein expression levels are expected if interactions at the DNA and RNA level, such as gene silencing, occur. Only small changes in protein expression levels were observed (see Table 1 for an example in seed). Taking this into account, as well as the inherent variability of plants, the observed small changes do not indicate the occurrence of interactions between the two transformation events in cotton MON 15985.

²⁷ Technical dossier, Section D2(b).

²⁸ Additional information: 05/11/2013.

²⁹ Additional information: 05/11/2013.

³⁰ Technical dossier, Section D3; additional information: 14/09/2012.

Table 1: Protein expression levels in cotton MON 15985, MON 531 and MON 15947 seed (µg/g fresh weight)

Year	Event	Cry1Ac	NPTII	Cry2Ab2	β-D-Glucuronidase
1998	MON 15985	3.35 (0.63) 2.21–4.84	10.8 (1.2) 8.88–13.2	43.2 (5.7) 31.8–50.7	58.8 (13.0) 37.2–82.3
		3.22 (0.77) 1.50–4.46	9.92 (2.19) 3.81–12.6	< LOD	< LOD
2001	MON 15985	1.6 (0.23) 1.3–1.9	5.5 (0.59) 4.8–6.2	44 (10) 34–60	46 (13) 27–59
		1.7 (0.079) 1.6–1.8	5.2 (0.5) 4.7–6.0	< LOD	< LOD
	MON 531	< LOD	< LOD	46 (7.6) 35–56	40 (9.5) 25–50
		< LOD	< LOD	< LOD	< LOD

Each value is represented as mean with standard deviation (in brackets) and range. LOD, limit of detection. Cotton MON 15947 derives from genetic segregation of cotton MON 15985.

3.1.5. Inheritance and stability of inserted DNA³¹

The genetic stability of the MON 531 and MON 15947 inserts was investigated by Southern analysis. The presence of the internal sequence and the flanking regions of the functional insert in MON 531 and of the flanking regions in MON 15947 indicates stable inheritance over several generations. The non-functional insert of the MON 531 event was not retained in the backcrossed lines³².

The expected inheritance ratio for the Cry2Ab2 protein was observed over several selfed generations and over successive backcross generations, indicating the presence of a stable single Mendelian locus. The phenotypic stability of Cry1Ac, Cry2Ab2, NPTII and β-D-glucuronidase expression was shown by ELISA measurements of the proteins in leaves and seeds of plants cultivated from several generations in different locations.

The possibility of a lack of co-inheritance of MON 531 and MON 15947 inserts in seeds derived from cotton MON 15985 cannot be excluded. However, the EFSA GMO Panel is of the opinion that, even though plants containing the MON 15947 insert have not been assessed as a single event (with the exception of expression data provided as additional information), plants containing only the MON 15947 insert would not give rise to an issue that would require further investigations. Furthermore, cotton is predominantly a self-pollinator, and cotton MON 15985, as assessed in this application, is homozygous for both inserts³³. Therefore, the produced and imported cottonseed of this GM cotton will contain all traits, and segregants are expected at only very low frequency.

Molecular characterisation data gave no indication of interaction between the combined MON 531 and MON 15947 inserts, and therefore did not identify issues that would require further investigations.

3.2. Conclusion

The molecular characterisation data establish that cotton MON 15985 contains two inserts containing the *cry1Ac*, *nptII*, *cry2Ab2* and *uidA* expression cassettes. Bioinformatic analyses of the ORFs spanning the junction sites within the inserts or between the inserts and genomic DNA did not give rise to safety issues. The stability of the inserted DNA and the expression of newly introduced proteins was confirmed over several generations. Protein levels were obtained and reported adequately. The potential impacts of the protein levels quantified in field trials carried out in the USA are assessed in the sections on food/feed safety assessment (Section 5) and ERA (Section 6).

³¹ Technical dossier, Section D5.

³² Additional information: 19/05/2009.

³³ Technical dossier, Section A6.

4. Comparative analysis

4.1. Evaluation of relevant scientific data

4.1.1. Summary of the previous evaluation of event MON 531

Compositional data for cotton MON 531 and its conventional counterpart (1992 and 1993: Coker 312; 1999: DP5415) were generated in field trials carried out in the USA in 1992, 1993 and 1999. The field trials performed in 1999 included, in addition to cotton MON 531 and its conventional counterpart, non-GM commercial cotton varieties. Cottonseed produced in 1993 was processed into toasted meal and refined cottonseed oil fractions and analysed for composition. Significant differences in cottonseeds were observed for myristic acid, stearic acid and oleic acid (1992), glutamic acid, valine, methionine, isoleucine, tyrosine, lysine and histidine (1993) and total fat, carbohydrates, palmitic acid, linoleic acid, calcium and iron (1999). However, these differences were not consistent and were found for only some growing seasons.

Information on agronomic performance and phenotypic characteristics of cotton MON 531 was derived from field trials performed in 1998 and 1999 in the USA. These studies showed significantly more cracked bolls in cotton MON 531 than in its conventional counterpart, possibly related to minor differences in insect damage. Other agronomic or phenotypic characteristics did not differ between cotton MON 531 and its conventional counterpart.

The analyses carried out on cotton MON 531, its conventional counterpart and other non-GM cotton varieties indicated that cotton MON 531 did not show any compositional, phenotypical or agronomical differences from its conventional counterpart that would lead to a need for further assessment. The comparative analysis of cotton MON 531 therefore provided no indication of unintended effects resulting from the genetic modification that would give rise to a safety concern (EFSA GMO Panel, 2011b).

4.1.2. Choice of comparator and production of material for the comparative assessment³⁴

Cotton MON 15985 was compared with its conventional counterpart cotton, DP50, during field trials in the USA in the years 1998, 1999 and 2007. The results of the studies carried out in 1998 and 1999 have been published (Hamilton et al., 2004).

Table 2: Overview of comparative assessment studies with cotton MON 15985

Study focus	Endpoints	Study details	Conventional counterpart	Non-GM cotton varieties
Agronomic and phenotypic characteristics and/or composition of harvested seeds	Various endpoints (see Sections 4.1.3 and 4.1.4)	1998, eight locations in the USA ^{(a), 35}	1 (DP50)	8
		1999, six locations in the USA ^(b)	1 (DP50)	15
		2007, five locations in the USA ³⁶	1 (Giza-90)	8

(a): In addition, the parental line MON 531 was also included.

(b): Field trials were used only for the compositional analysis.

In the 1999 field trials, cotton MON 15985 (with a *G. hirsutum* background) and its conventional counterpart, DP50, were grown in six locations in the USA. Since cotton MON 15985 was established

³⁴ Technical dossier, Section D7.2; additional information: 18/01/2010 on EFSA-GMO-RX-MON15985.

³⁵ Technical dossier, Section D7.2.

³⁶ Additional information: 12/03/2012.

by re-transformation of callus tissue derived from cotton MON 531 in a *G. hirsutum* DP50 genetic background, and subsequently backcrossed with DP50, the EFSA GMO Panel considers DP50 as a suitable conventional counterpart for cotton MON 15985. At all locations, two to four non-GM commercial cotton varieties were included (in total, 15 non-GM varieties³⁷). At each site, all test materials, were planted using a randomised complete block design with four replications.

In the 2007 field trials, cotton MON 15985 (in a *G. barbadense* background) and its conventional counterpart, Giza-90 (with similar genetic background), were grown in a randomised complete block design with three replicates at five locations, representing the major cotton-growing areas of the USA. In addition, eight different non-GM commercial varieties (four at each site) were included in the field trials. Acid-delinted cottonseed from all test material was used for the compositional analysis.

The application also included reports from a study performed in the USA in 1998 with cotton MON 15985 and its conventional counterpart, DP50, the parental line, MON 531 and various commercial cotton varieties grown in eight locations for analysis of composition of seed and processed seed fractions³⁸, the outcomes of which are further discussed in Section 5.1.2, as well as phenotypic and agronomic characteristics. The starting seed material for MON 15985 and the conventional counterpart used in this study were produced under different environmental conditions, which may have affected seed quality³⁹. Given that differences in seed quality, unrelated to the genetic transformations, would affect the outcome of the comparative assessment, the EFSA GMO Panel considers that data obtained from the 1998 study cannot be used to identify potential effects of the genetic modification.

At the request of the EFSA GMO Panel, the applicant provided additional information on field trials carried out in Brazil and India⁴⁰. In Brazil, cotton MON 15985 was compared with the conventional counterpart and various commercial varieties in three locations during the 2005/2006 growing season. In India, the agronomic and phenotypic characteristics of several varieties containing the MON 15985 and MON 531 events and the corresponding non-GM varieties were studied for agronomic and phenotypic characteristics during two years (2002, 2003) and for compositional characteristics during a single year (2002). These studies were considered as only confirmatory owing to the limited number of locations in Brazil and also the limited description of the field trial design and the lack of appropriate statistical analysis for the Indian trials. The current assessment focuses on data obtained from the 1999 and 2007 field studies.

4.1.3. Agronomic traits and GM phenotype⁴¹

In the 2007 field trials, 42 agronomic and phenotypic characteristics⁴² were compared between MON 15985 and its conventional counterpart Giza-90. In the combined-site analysis significant

³⁷ Including also the parental non-GM line DP50 (from a different seed lot than the conventional counterpart DP50).

³⁸ Seeds from nine commercial lines, including four non-GM and five GM cotton lines, were supplied as reference lines for the compositional comparison but these data were not used by the EFSA GMO Panel because the lines had been grown in field trials other than those for the GMO and the conventional counterpart, during the same season.

³⁹ Additional information: 05/11/2012.

⁴⁰ Additional information: 11/11/2013.

⁴¹ Technical dossier, Section D7.4.

⁴² Average number of immature seeds/boll, average number of mature seeds/boll, average number of seeds/boll, average number of vegetative bolls/plant, average total number of main stem nodes/plant, average weight per boll (g), boll retention at P1 (position 1) of nodes 4–9 (%), boll retention at P1 of nodes 10–14 (%), boll retention at P1 of nodes 15–19 (%), boll retention at P1 of nodes 20–26 (%), boll retention at P2 (position 2) of nodes 4–9 (%), boll retention at P2 of nodes 10–14 (%), boll retention at P2 of nodes 15–19 (%), boll retention at P2 of nodes 20–26 (%), fibre elongation (%), fibre length (inches), fibre micronaire (mic units), fibre strength (g/tex), fibre uniformity (%), height (inches), nodes above cracked boll (NACB) observation 1 (no of nodes), NACB observation 2 (no of nodes), NACB observation 3 (no of nodes), nodes above white flower (NAWF) observation 1 (no of nodes), NAWF observation 2 (no of nodes), NAWF observation 3 (no of nodes), percentage of total bolls that are abnormal (%), plant height at four weeks (inches), plant height at eight weeks (inches), plant vigour at four weeks (rating 1–9), plant vigour at eight weeks (rating 1–9), seed cotton yield (pounds/acre), seed index of 100 ginned seed (g), stand count at two weeks, stand count at four weeks, total abnormal position 1 (P1) bolls, total abnormal position 2 (P2) bolls, total bolls on plant, total normal P1 bolls, total normal P2 bolls, total P1 bolls, total P2 bolls.

differences were observed for fibre elongation (11.0 % (MON 15985) vs. 11.6 % (Giza-90)), fibre uniformity (84.0 (MON 15985) vs. 82.6 % (Giza-90)) and fibre height (3.07 cm (MON 15985) vs. 2.97 cm (Giza-90)). The mean values for both cotton MON 15985 and its conventional counterpart were outside the range of the commercial non-GM varieties. However, the observed differences fell within the range of values for conventional *G. barbadense* reported in the literature (Percy and Turcotte, 1992)⁴³.

The EFSA GMO Panel could not complete the assessment of the agronomic and phenotypic characteristics of cotton MON 15985 on the basis of data provided (a single season and fewer than eight sites (EFSA, 2006a; EFSA GMO Panel, 2011a)). Therefore, the EFSA GMO Panel could not conclude on the potential occurrence of unintended effects based on the outcome of the agronomic and phenotypic analysis.

The relevance for the ERA is further discussed in Section 6.1.2.1.

4.1.4. Compositional analysis⁴⁴

The design of the field trials to produce material for the comparative compositional assessment of cotton MON 15985 is summarised in Table 2 (see Section 4.1.2).

In the field trials in 1999, seeds of cotton MON 15985, its conventional counterpart and the commercial non-GM cotton varieties were assessed for 49 parameters^{45,46}. The statistical analysis of compositional data from 1999 identified significantly increased levels of dihydrosterculic acid, calcium and the fatty acids myristic acid, stearic acid and arachidic acid, as well as decreased levels of gossypol (free and total), the fatty acids palmitic acid and linoleic acid, copper, iron, phosphorus and potassium in cotton MON 15985 (Table 3).

In the 2007 field trials, acid-delinted seeds of cotton MON 15985, its conventional counterpart and the commercial non-GM cotton varieties were assessed for 65 parameters⁴⁷. More than 50 % of the analytical values for 13 fatty acids were below the limit of quantification (LOQ) and were not included in the statistical analyses⁴⁸. Therefore, only 52 endpoints were statistically analysed. Significantly increased levels of myristic acid, palmitoleic acid and α -tocopherol, as well as decreased levels of palmitic acid, were found in cotton MON 15985 (Table 3).

⁴³ <http://r0.unctad.org/infocomm/anglais/cotton/sitemap.htm#site>

⁴⁴ Technical dossier, Section D7.1; additional information, 18/01/2010.

⁴⁵ The following parameters were analysed: moisture, protein, total fat, ash, carbohydrates, calories, crude fibre, total and free gossypol, calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, zinc, sterculic acid, malvalic acid, dihydrosterculic acid, behenic acid, arachidic acid, linolenic + gamma-linolenic acid, linoleic acid, stearic acid, oleic acid, palmitoleic acid, palmitic acid, pentadecanoic acid, myristic acid, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine, tryptophan.

⁴⁶ Although not endogenously produced by cotton, the seeds were also analysed for aflatoxins.

⁴⁷ The following parameters were analysed: protein, total fat, ash, moisture and carbohydrate (calculated), fibre fractions (acid detergent fibre (ADF), neutral detergent fibre (NDF) and total dietary fibre (TDF), crude fibre), 9 minerals, 18 amino acids, 25 fatty acids, α -tocopherol, anti-nutrients (total gossypol, free gossypol) and calories (calculated).

⁴⁸ 10:0 Capric acid, 12:0 lauric acid, 14:1 myristoleic acid, 15:0 pentadecanoic acid, 15:1 pentadecenoic acid, 17:0 heptadecanoic acid, 17:1 heptadecenoic acid, 18:3 gamma-linolenic acid, 20:1 eicosenoic acid, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, 20:4 arachidonic acid, 8:0 caprylic acid.

Table 3: Compositional endpoints in cotton seeds harvested from field trials with cotton MON 15985 and its conventional counterpart (DP50 in 1999 and Giza-90 in 2007) for which a statistically significant difference was observed in the across-site analysis

Parameter	Conventional counterpart	MON 15985	Commercial non-GM varieties (range min.–max. values)
Field trials in 1999			
14:0 Myristic acid (% total FA)	0.99 ± 0.06	1.12 ± 0.06	0.55–1.28
16:0 Palmitic acid (% total FA)	25.08 ± 0.33	24.84 ± 0.33	21.23–26.45
18:0 Stearic acid (% total FA)	2.19 ± 0.053	2.49 ± 0.05	1.99–2.48
18:2 Linoleic acid (% total FA)	53.39 ± 0.73	53.08 ± 0.73	49.90–56.88
20:0 Arachidic acid (% total FA)	0.28 ± 0.01	0.29 ± 0.01	0.25–0.33
Dihydrosterculic acid C19 (% total FA)	0.15 ± 0.01	0.17 ± 0.01	0.13–0.24
Calcium (% DW)	0.13 ± 0.01	0.13 ± 0.01	0.10–0.16
Copper (mg/kg DW)	7.07 ± 0.91	6.70 ± 0.91	3.54–11.14
Iron (mg/kg DW)	49.96 ± 1.63	46.58 ± 1.64	40.58–56.54
Phosphorus (% DW)	0.69 ± 0.02	0.65 ± 0.02	0.60–0.84
Potassium (% DW)	1.09 ± 0.02	1.06 ± 0.02	0.98–1.14
Free gossypol (% DW)	0.87 ± 0.04	0.82 ± 0.04	0.53–1.20
Total gossypol (% DW)	0.99 ± 0.05	0.92 ± 0.05	0.57–1.42
Field trials in 2007			
14:0 Myristic acid (% total FA)	0.70 ± 0.03	0.79 ± 0.03	0.49–0.78
16:0 Palmitic acid (% total FA)	23.22 ± 0.57	22.35 ± 0.56	20.45–24.35
16:1 Palmitoleic acid (% total FA)	0.77 ± 0.02	0.82 ± 0.02	0.60–0.81
α-Tocopherol (mg/kg DW)	63.72 ± 11.24	77.71 ± 11.07	29.64–99.98

Values are reported on a dry-weight basis. The mean values with standard error are given.
DW, dry weight; FA, fatty acids.

For all parameters showing differences, the average values fell within the range of commercial non-GM cotton varieties grown in the same field trials, with the exception of stearic acid in 1999, palmitoleic acid in 2007 and myristic acid in 2007 (Table 3). Given the magnitude of these changes and the characteristics of these endpoints, the EFSA GMO Panel concludes that compositional data give no indication that the genetic modification induces unintended effects for which further assessment is needed.

The EFSA GMO Panel considered the total set of compositional data supplied and the outcome of the statistical analysis comparing cotton MON 15985, its conventional counterparts and the set of non-GM cotton varieties in the field trials carried out in 1999 and 2007. The EFSA GMO Panel concludes that compositional data give no indication that the genetic modification induces unintended effects for which further assessment is needed.

4.2. Conclusion

No differences in compositional data between cotton MON 15985 and its conventional counterpart necessitating further assessment with regard to safety were identified. The EFSA GMO Panel could not complete the assessment of the agronomic and phenotypic characteristics of cotton MON 15985 on the basis of data provided (a single season and fewer than eight sites (EFSA, 2006a; EFSA GMO Panel, 2011a)). Therefore, the EFSA GMO Panel could not conclude on the potential occurrence of unintended effects based on the outcome of the agronomic and phenotypic analysis.

5. Food/feed safety assessment

5.1. Evaluation of relevant scientific data

5.1.1. Summary of the previous evaluation of event MON 531

Cotton MON 531 expresses the Cry1Ac and NPTII proteins. *E. coli*-produced Cry1Ac and NPTII proteins were used for the safety studies after it had been demonstrated that they are 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 were rapidly degraded by proteolytic enzymes in *in vitro* studies, 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 not expected to have any adverse effects on human and animal health in the context of their intended uses (EFSA GMO Panel, 2011b).

5.1.2. Effect of processing⁴⁹

Refined oil (i.e. bleached and deodorised oil) was produced from the cottonseeds harvested in the 1998 season and analysed for its contents of fatty acids, α -tocopherol and gossypol, whilst toasted meal was analysed for gossypol only. Since data from the 1998 field trial were rejected for the comparative assessment, those results were not further considered.

No differences in compositional data of cotton MON 15985 and its conventional counterpart necessitating further assessment with regard to safety were identified except for the introduced trait (see Section 4.2). The EFSA GMO Panel considered that the effect of processing on cotton MON 15985 is not expected to be different from the effect on conventional cotton varieties.

5.1.3. Toxicology⁵⁰

Cotton MON 15985 expresses four new proteins: Cry1Ac, NPTII, Cry2Ab2 and GUS E377K. Cry1Ac and NPTII proteins have been previously assessed for safety in connection with the risk assessment of cotton MON 531 (EFSA GMO Panel, 2011b), from which MON 15985 was obtained by retransformation. In addition, the safety of NPTII has previously been assessed by the EFSA GMO Panel in other GM crops (EFSA, 2004a, b, 2006c; EFSA GMO Panel, 2010c, 2012). The safety of a Cry2Ab2 protein with an almost identical amino acid sequence also has been previously assessed by the EFSA GMO Panel for maize MON 89034 (EFSA, 2008).

5.1.3.1. Proteins used for safety assessment

Given the low expression levels of the Cry2Ab2 protein in the GM crop and the consequent difficulty in extracting sufficient protein from the GM cotton, the protein was produced in a GM *B. thuringiensis* strain, EG7699. For equivalence testing, plant-derived Cry2Ab2 protein was obtained from both cotton MON 15985 and a second cotton, MON 15813, obtained using the same transformation vector as for MON 15985. The MON 15813 source was chosen because of easy extraction of the Cry2Ab2 protein in sufficient amounts for experimental purposes to corroborate equivalence testing⁵¹. Proteins were purified by chromatographic methods. Cry2Ab2 from leaves of MON 15985 and MON 15813, and from *B. thuringiensis*, displayed immunoreactive bands corresponding to proteins of the same molecular size (62 to 63 kDa). In addition, Cry2Ab2 from MON 15813 and its bacterial analogue both reacted negatively in the glycosylation assay and had similar half-minimal effective concentration (EC₅₀) values in the insect bioassay on larvae of *Helicoverpa zea*. Cry2Ab2 proteins from cotton MON 15813 and from *B. thuringiensis* were further characterised by matrix-assisted laser

⁴⁹ Technical dossier, Section D7.6.

⁵⁰ Technical dossier, Section D7.8; additional information: 11/11/2013.

⁵¹ Holleshack et al. (1999).

desorption/ionisation-time-of-flight (MALDI-TOF) after tryptic digestion by reverse phase high-performance liquid chromatography (HPLC) followed by mass spectrometry (quadrupole-time-of-flight (Q-TOF)) of column eluates containing separated peptides, and by N-terminal sequencing of the peptides in two selected fractions collected after elution. The peptides thus identified corresponded to the cleavage products derived from the sequence of the Cry2Ab2 protein. The EFSA GMO Panel accepts the use of the microbe-derived Cry2Ab for safety tests.

The GUS E377K protein expressed in cotton MON 15985 was extracted from cottonseeds and purified by ion exchange chromatography. The identity of the purified protein was determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by Western blotting, N-terminal sequencing of four peptide bands observed on SDS-PAGE and by MALDI-TOF after tryptic digestion. In addition, the purified protein preparation was tested for β -glucuronidase activity. Of the protein bands observed in the SDS-PAGE, two, with apparent molecular weights of 72 and 148 kDa, were identified as GUS proteins, whilst another band with apparent molecular weight of 52 kDa was identified as alanine aminotransferase. A fourth faint band (36 kDa) could not be identified. The two bands that were identified as GUS were also reactive in Western blots. The peptides identified through MALDI-TOF mass spectrometry of the trypsin cleavage products of these two bands corresponded to the sequence of GUS E377K, indicating that the protein in the higher-molecular-weight band, with apparent molecular weight of 148 kDa, was probably a dimer of the monomer in the band with an apparent weight of 72 kDa. The protein preparation also exhibited β -glucuronidase activity. The GUS E377K protein expressed in cotton MON 15985 is not glycosylated.

5.1.3.2. Toxicological assessment of newly expressed proteins in cotton MON 15985

The GUS E377K protein expressed in cotton MON 15985 is a β -glucuronidase, a family of enzymes widely distributed in nature, including humans. The particular enzyme under scrutiny is derived from *E. coli* K12, a common inhabitant of the gastrointestinal tract in vertebrates.

(a) Acute toxicity

In an acute oral toxicity study in CD-1 mice, the Cry2Ab2 protein from *B. thuringiensis* did not induce adverse effects up to the maximum dose of 1 450 mg/kg body weight. No adverse effects were seen for the GUS protein at the highest dose of 100 mg/kg body weight tested under the same conditions.

The EFSA GMO Panel considers that acute toxicity testing of the newly expressed proteins is of little value for the risk assessment of the repeated human and animal consumption of food and feed derived from GM plants.

(b) In vitro degradation by proteolytic enzymes

The resistance to degradation by pepsin of the Cry2Ab2 and of the GUS E377K proteins was investigated in solutions at pH $\approx$ 1.2 in two independent studies. The integrity of the test proteins in probes taken at various time points was analysed by SDS-PAGE followed by protein staining. In the case of Cry2Ab2, the integrity of the protein was also analysed by Western blotting. The Cry2Ab2 protein was degraded by pepsin within 15 seconds. The GUS E377K full-length protein was degraded by pepsin within 15 seconds. Proteolytic fragments of GUS E377K were reported to be degraded by pepsin within four minutes.

(c) Bioinformatic studies

Bioinformatic analyses of the amino acid sequences of the Cry1Ac, NPTII, Cry2Ab2 and GUS E377K proteins in cotton MON 15985 revealed no significant similarities to known toxic proteins⁵².

⁵² Technical dossier, Section D7.8.1; additional information: 14/09/2012 and 11/11/2013.

5.1.3.3. Toxicological assessment of new constituents other than proteins

No new constituents, other than the Cry1Ac, Cry2Ab2, NPTII and GUS E377K proteins, are expressed in cotton MON 15985 and no biologically relevant changes in the composition of cotton MON 15985 were detected in the comparative compositional analysis (see Section 4.1.4).

5.1.3.4. Toxicological assessment of the whole GM food/feed⁵³

(a) Sub-chronic toxicity study

The applicant provided a repeated-dose 90-day feeding study in rats with ground cottonseed of MON 15985, the conventional counterpart (DP50) and six non-GM commercial cotton varieties.

Twenty rats (CrI:CD®(SD)IGS BR) of each sex received one of 10 experimental diets. Two of these diets contained ground cottonseed of MON 15985, PCR-confirmed, at inclusion levels of 2 % and 5 % (w/w). Two other diets contained the corresponding amounts of control ground cottonseed DP50, and the six remaining diets 5 % (w/w) ground cottonseed of commercial non-GM cotton varieties⁵⁴. The test material was added to a standard rodent diet.

Feed intake, body weight and clinical abnormalities were recorded. Interim (week 5) and terminal (week 14) clinical chemistry, haematology and urine analyses were performed on 10 animals per sex/group. Post-mortem measurements included organ weight determinations, gross pathology and histopathology on control and high-dose rats.

Two mortalities occurred during the experiment, one in the 5 % control group and the other in one of the six reference groups. Feed intake and body weight gain were comparable in the test and the control group. Several significant differences were observed between the test and the control group in haematology, clinical chemistry and urine analyses. These differences were not dose related, occurred at only one time point and in one sex and/or fell within the range of reference groups. No significant differences in absolute and relative organ weights were observed. Macroscopic examination and histopathology of selected tissues and organs revealed no test-substance-related changes.

The EFSA GMO Panel concludes that there were no indications of adverse effects after administration of diets containing ground cottonseed of MON 15985 up to the 5 % inclusion level.

(b) Animal feeding study

The applicant provided a feeding study⁵⁵ with channel catfish (*Ictalurus punctatus*) fed diets containing meal from GM cotton MON 15985, the conventional counterpart (DP50), the parental GM commercial line MON 531 (DP50B), MON 15813 (another GM cotton line expressing the Cry2Ab2 protein) and two commercial non-GM cotton reference varieties (ST474, DP1266) at a 20 % inclusion level. For each treatment, 100 catfish were used, divided over 5 aquaria with 20 fish each. Feed consumption was measured and behavioural observations were made daily, whereas body weights were measured only at the beginning of the experiment, after four weeks and at the end of the experiment of eight weeks. After the experiment, five fish per aquarium were used to prepare fillets, which were pooled for compositional analysis (moisture, crude protein, crude fat, ash), yielding five pooled fillet samples per treatment group.

The feed consumption, weight gain, feed conversion ratio, visceral fat (% of body weight), fillet composition, survival and behaviour of fish fed the diet containing meal of cotton MON 15985 did not significantly differ from those of fish fed the other diets. Consequently, this experiment produced no evidence of unintended effects.

⁵³ Technical dossier, Section D7.8.4.

⁵⁴ Reference control lines: Chaco 5201, Guazuncho, Pora, DP5415, DP5690 and ST474.

⁵⁵ Technical dossier, Section D7.10; additional information: 05/11/2012.

5.1.4. Allergenicity

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

5.1.4.1. Assessment of allergenicity of the newly expressed proteins

A weight of evidence approach is followed, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (Codex Alimentarius, 2009; EFSA, 2006a; EFSA GMO Panel, 2010b).

The genes coding for the newly expressed Cry1Ac, Cry2Ab2, NPTII and GUS E377K proteins in cotton MON 15985 derive from *B. thuringiensis* and *E. coli*, which are not considered to be common allergenic sources.

Bioinformatic analyses⁵⁶ of the amino acid sequences of the Cry1Ac, Cry2Ab2, NPTII and GUS E377K proteins using the criterion of 35 % identity in a window of 80 amino acids revealed no significant similarities to known allergens. In addition, the applicant performed analyses searching for matches of eight contiguous identical amino acid sequences between these newly expressed proteins and known allergens, which confirmed the outcome of the above-mentioned bioinformatic analyses showing no similarities to known allergens.

The studies on resistance to degradation by proteolytic enzymes presented in the current application have been described in Section 5.1.3.2.

The EFSA GMO Panel has previously evaluated the safety of the Cry1Ac, Cry2Ab2 and NPTII proteins in the context of several other applications and no concerns about allergenicity were identified (e.g. EFSA, 2004a, b, 2006c, 2008; EFSA GMO Panel, 2010c, 2011b, 2012).

The EFSA GMO Panel considered that there are no indications that the newly expressed Cry1Ac, Cry2Ab2, NPTII and GUS E377K proteins in cotton MON 15985 may be allergenic under the intended conditions of use. In addition, based on current knowledge and since none of the newly expressed proteins showed allergenicity, no concerns regarding the mixture of these newly expressed proteins in cotton MON 15985 affecting allergenicity are expected.

With regard to adjuvanticity, Bt proteins have been suggested to possess adjuvant activity, based on animal studies on Cry1Ac (e.g. Vazquez-Padron et al., 1999; Moreno-Fierros et al., 2003; Rojas-Hernandez et al., 2004). However, at present, there is no evidence for Bt protein adjuvanticity of safety concern among the GM plants assessed so far by the EFSA GMO Panel (EFSA, 2009a; EFSA GMO Panel, 2011b, c). In relation to the NPTII and GUS E377K proteins, no concerns regarding adjuvanticity were identified in the scientific literature or in the data provided by the applicant. The expression levels of the newly expressed proteins in cotton MON 15985 are similar to those in cotton MON 531 and MON 15947 (see Section 3.1.4). In addition, there is no information available on the structure or function of the newly expressed Cry1Ac, Cry2Ab2, NPTII and GUS E377K proteins that would suggest an adverse adjuvant effect of their mixture in cotton MON 15985 under the intended conditions of use.

⁵⁶ Technical dossier, Section D7.9.1; additional information: 14/09/2012 and 11/11/2013.

5.1.4.2. Assessment of allergenicity of the whole GM plant

Cotton is not considered to be a common allergenic food (OECD, 2009)⁵⁷. A few cases of food allergy to cottonseed have been reported (Atkins, 1988; Malanin and Kalimo, 1988; O'Neil and Lehrer, 1989; de Olano et al., 2009; Mane et al., 2013), all of which were related to foods in which cottonseed flour was the offending ingredient. However, the main cottonseed product in human food, industrially processed cottonseed oil, is highly purified and contains negligible levels of proteins. Furthermore, the protein level in cellulose from cottonseed linters for food use is very low.

In the context of this application, and considering the data from the molecular characterisation, the compositional analysis and the assessment of the newly expressed proteins, the EFSA GMO Panel identified no indications of safety concern regarding the overall allergenicity of cotton MON 15985.

5.1.5. Nutritional assessment of GM food/feed

The intended trait of cotton MON 15985 is insect resistance, with no intention to alter nutritional parameters. The outcome of the compositional analysis (see Section 4.1.4) confirmed the nutritional adequacy of the food and feed products (cottonseed, refined oil and toasted cottonseed meal) derived from cotton MON 15985. The introduction of these products into the food and feed supply is, therefore, not expected to have any nutritional impact, similar to its conventional counterpart and non-GM cotton varieties.

The nutritional similarity of cotton MON 15985 to commercial non-GM cotton varieties, indicated by compositional data, was corroborated by a study with MON 15985 in catfish⁵⁸ and a number of published feeding studies with this cotton in dairy cattle (Castillo et al., 2004), chickens (Mandal et al., 2004) and quails (Hamilton et al., 2004).

The EFSA GMO Panel concludes that the data provided support the view that diets formulated with cottonseed meal derived from MON 15985 are as nutritious as those formulated with cottonseed meal derived from commercial non-GM cotton varieties.

5.1.6. Post-market monitoring of GM food/feed

The EFSA GMO Panel considers that post-market monitoring of GM food/feed from cotton MON 15985 is not necessary.

5.2. Conclusion

The newly expressed proteins in cotton MON 15985 do not give rise to safety concerns for human and animal health, since no adverse effects in the available studies were observed and no structural similarities to known toxins were detected. Similarly, the EFSA GMO Panel did not identify indications of safety concerns regarding allergenicity or adjuvanticity with the newly expressed proteins in cotton MON 15985. The cotton MON 15985 is as nutritious as its conventional counterpart and non-GM commercial varieties.

The EFSA GMO Panel concludes that cotton MON 15985 is as safe and nutritious as its conventional counterpart and that it is unlikely that the overall allergenicity of the whole plant is changed.

⁵⁷ Directive 2007/68/EC of the European Parliament and of the Council of 27 November 2007 amending Annex IIIa to Directive 2000/13/EC of the European Parliament and of the Council as regards certain food ingredients. OJ L 310, 27.11.2007, p. 11–14.

⁵⁸ Technical dossier, Section D7.10; additional information: 05/11/2012.

6. Environmental risk assessment and monitoring plan

6.1. Evaluation of relevant scientific data

The scope of applications EFSA-GMO-UK-2008-57 and EFSA-RX-MON15985 includes *G. hirsutum* and *G. barbadense*⁵⁹ and covers cotton MON 15985 for food and feed uses, import and processing, food additives produced from cotton MON 15985 and feed produced from cotton MON 15985 (feed materials and feed additives) but does not include cultivation⁶⁰. Considering the intended uses of cotton MON 15985, the ERA is concerned mainly with ingestion by an animal leading to exposure of bacteria within its gastrointestinal tract, and to exposure of soil bacteria from the faecal material of such an animal, and with the accidental release into the environment of viable seeds of cotton MON 15985 (e.g. during transport and/or processing).

Cotton MON 15985 has been developed by re-transformation via a biolistic system of cotton event MON 531 (see Sections 3.1.2 and 3.1.4) with the event MON 15947 to confer resistance to certain lepidopteran pests by the expression of the *B. thuringiensis*-derived Cry1Ac and Cry2Ab2 proteins, respectively.

6.1.1. Evaluation of transformation events in cotton MON 15985

In its previous scientific opinions, the EFSA GMO Panel was of the opinion that the single cotton events MON 531 is as safe as its conventional counterpart, and that the placing on the market of cotton MON 531 for food and feed uses, import and processing is unlikely to have an adverse effect on human or animal health, or on the environment (EFSA GMO Panel, 2011b). Furthermore, PMEM plans for cotton MON 531, including general surveillance, were proposed by the applicants and considered in line with the EFSA GMO Panel scientific opinion on PMEM (EFSA, 2006d; EFSA GMO Panel, 2011d).

Event MON 15947 was not previously risk assessed since it was used only in the re-transformation process. The event MON 15947 segregates as a single Mendelian locus, as demonstrated by the applicant⁶¹.

A segregant line harbouring event MON 15947 only has been derived from the original re-transformant MON 15985 for regulatory purposes and, in particular, to analyse protein expression data. This information was submitted by the applicant and assessed by the EFSA GMO Panel⁶².

Cotton is predominantly a self-pollinator and cotton MON 15985, as assessed in this application, is homozygous for both inserts⁶³. Therefore, the produced and imported cottonseed of this GM cotton will contain all traits, and segregants are expected at only very low frequency. Should segregation of MON 531 and MON 15947 events occur, its possible implications are assessed below.

6.1.2. Environmental risk assessment

6.1.2.1. Unintended effects on plant fitness due to the genetic modification⁶⁴

Gossypium herbaceum is a highly domesticated crop which has been grown in Southern Europe since the 19th century, giving rise to feral plants which can occasionally be found in the same area (Todaro 1917; Davis, 1967). From recent available data, it is possible to see that, in the EU, cotton is cultivated in Greece and Spain (EUROSTAT, 2013). The main cultivated cotton species (*G. hirsutum*), which has been present in Southern Europe since the 19th century, is an annual self-pollinator. In the absence of insect pollinators (such as wild bees, honeybees, bumblebees), cotton flowers are self-pollinating,

⁵⁹ Clarification from the applicant: 15/09/2010.

⁶⁰ Application summary; extension of scope by the applicant: 18/03/2013.

⁶¹ Technical dossier, Section D5.

⁶² Additional information: 14/09/2012.

⁶³ Technical dossier, Section A6.

⁶⁴ Technical dossier, Sections D4 and 7.4; additional information: 05/11/2012 and 12/03/2013.

but when these pollinators are present low frequencies of cross-pollination can occur (McGregor, 1959; Moffett and Stith, 1972; Moffett et al., 1975; Van Deynze et al., 2005).

Pollen and cottonseed dispersal are potential sources of vertical gene flow to cross-compatible wild cotton relatives, other cotton varieties and to occasional feral cotton plants. However, in Europe, there are no cross-compatible wild relatives with which cotton can hybridise. Because cotton pollen is very large (120–200 µm), heavy and sticky, wind-mediated dispersal of pollen to cross-pollinate other cotton varieties is considered negligible (Vaissiere and Vinson, 1994). In addition, cross-pollination percentages rapidly decrease with increasing distance from the pollen source (Umbeck et al., 1991; Kareiva et al., 1994; Llewellyn and Fitt, 1996; Xanthopoulos and Kechagia, 2000; Zhang et al., 2005; Van Deynze et al., 2005, 2011; Hofs et al., 2007; Llewellyn et al., 2007; Heuberger et al., 2010).

Seeds are the only survival structures. However, seed-mediated establishment of cotton and its survival outside cultivation in Europe are mainly limited by a combination of absence of a dormancy phase, low competitiveness and susceptibility to diseases and cold climate conditions (Eastick and Hearnden, 2006). Even in regions where cotton is widely grown, such as Australia, the risk of GM cotton becoming feral along transportation routes, or a weed on dairy farms where raw cottonseed is used as feed, has been shown to be negligible (Addison et al., 2007). In arid areas where cotton is cultivated in Europe, adequate soil moisture is an additional factor affecting the survival of feral cotton seedlings. Since the limited data available do not indicate any relevant change in the general characteristics of cotton MON 15985 compared with its conventional counterpart, the inserted insect resistance trait is not likely to provide a selective advantage outside cultivation in Europe. If accidental spillage and subsequent release into the environment of cotton MON 15985 seeds occurs, cotton MON 15985 plants would have a selective advantage only under conditions of high infestation by susceptible lepidopteran species. Insect resistance against certain lepidopteran pests, such as cotton bollworm (CBW, *Helicoverpa armigera*), pink bollworm (PBW, *Pectinophora gossypiella*) and tobacco budworm (TBW, *Heliothis virescens*), provides a potential advantage in cultivation under infestation conditions, but plant survival is also limited by sensitivity to a range of other environmental factors. It is thus considered very unlikely that cotton MON 15985, or its progeny, will differ from other cotton varieties in their ability to survive until subsequent seasons or to establish feral populations under European environmental conditions.

The applicant presented in the application data gathered over a series of field trials conducted across eight locations in the USA in 1998, as described in Section 4.1.2. Information on phenotypic and agronomic characteristics was provided to assess the agronomic performance of cotton MON 15985 in comparison with its conventional counterpart, DP50. In particular, in the 1998 field trials, the comparative assessment was conducted comparing the event MON 15985 introgressed into the genetic background of the cotton Upland elite cultivar belonging to the *G. hirsutum* L. species; consequently, the event MON 15985 assessed in the 1998 field trials was also *G. hirsutum*. The 1998 field trials presented in the application were statistically re-analysed by the applicant at the request of the EFSA GMO Panel⁶⁵. The statistical analysis provided was conducted from analysis of data from only four sites (out of seven) because three of the sites did not have sufficient replicated entries⁶⁶. The agronomic and phenotypic analysis identified seven statistically significant differences (of 11 parameters tested) in the across location statistical analysis. Cotton MON 15985 had a higher stand count at 14 and 30 days after planting, a higher number of flowers at visits 3, 4, 5 and 6 during the flowering period and an increased yield than its conventional counterpart. Experimental data provided by the applicant showed that seed germination of cotton MON 15985 was in some cases significantly lower than that of its conventional counterpart. The applicant stated that the seed lots were grown under different environmental conditions and claimed that this may have affected seed quality⁶⁷. Since differences in starting seed quality would influence the outcome, the EFSA GMO Panel was not able to conclude on the data generated from these studies⁶⁸. In the additional information provided by the

⁶⁵ Additional information: 05/11/2012.

⁶⁶ Additional information: 05/11/2012.

⁶⁷ Additional information: 05/11/2012.

⁶⁸ Additional information: 05/11/2012.

applicant, data generated during the 2007 growing season in the USA from five sites were analysed⁶⁹. In this study, the MON 15985 event had been introgressed into the genetic background of Giza-90 used as the recurrent parent. Giza-90 is a Pima cotton variety, belonging to the species *G. barbadense* L. The number of backcrosses with the recurrent parent is expected to produce more than 99 % isogeneity between the MON 15985 and its conventional counterpart. The statistical analysis identified three phenotypic significant differences (of 42 parameters tested), all related to the characteristics of the fibres (elongation, uniformity and length). In the 2007 field trials, ecological interactions were also assessed, such as the response to abiotic stressors and data on diseases produced by fungi and arthropods; for these three categories 8, 10 and 9 endpoints were measured, respectively. The analyses of the ecological interactions revealed only one difference between MON 15985 and its conventional counterpart, related to the lower damage caused by PBW in the former. This difference was expected since the insect-protection trait expressed in MON 15985 is intended to control this pest.

In accordance with its guidance document on the ERA of GM plants (EFSA GMO Panel, 2010a), the EFSA GMO Panel follows a weight of evidence approach in collating and assessing appropriate information from various data sources (e.g. molecular and compositional data, available agronomic and phenotypic data from field trials performed by the applicant and the scientific literature) in order to assess the likelihood of unintended effects on the environment. The applicant provided molecular and compositional data that are assessed by the EFSA GMO Panel in Sections 3 and 4, respectively. In addition, the applicant presented and analysed agronomic and phenotypic data gathered from field trials with cotton MON 15895 introgressed into the *G. hirsutum* L. genetic background across four locations in the USA in 1998, and five locations in USA in 2007, with the MON 15895 introgressed into the *G. barbadense* L. genetic background. For each site in 1998 and 2007, information on phenotypic and agronomic characteristics was provided to assess the agronomic performance of cotton MON 15895 in comparison with the appropriate conventional counterpart (DP50 and Giza-90, respectively). However, as explained above, the 1998 field trials cannot be exploited to assess the potential effect of the introduced trait and/or the genetic modification in cotton MON 15985 on the agronomic performance compared with its conventional counterparts. In response to requests for further information, the applicant submitted the comparative analysis performed for regulatory applications in Brazil and India⁷⁰. The additional information provided has been assessed by the EFSA GMO Panel, but was deemed inappropriate owing to the limited number of locations in Brazil, the limited description of the field trial design for both Brazil and India and the lack of appropriate statistical analysis for the Indian trials. Therefore, the EFSA GMO Panel can base its assessment on only the field trials performed in 2007, which were conducted in one single growing season and at five locations. However, the assessment of the agronomic and phenotypic characteristics of cotton MON 15985 requires at least two seasons of data according to the applicable guidance document (EFSA, 2006a) (see Sections 4.1.2 and 4.2).

On the basis of the EFSA opinion on MON 531 (EFSA, 2011b) in which it was indicated that this single event does not show altered agronomic and phenotypic performance, as well as the information available in the current opinion, the EFSA GMO Panel is of the opinion that, in case of segregation, it is unlikely that MON 15947 will express altered agronomic and phenotypic performance.

In addition to the data presented by the applicant, the EFSA GMO Panel is not aware of any scientific report of increased fecundity, persistence (volunteerism) or ferality of GM cotton in regions where it is cultivated (Eastick and Hearnden, 2006; Bagavathiannan and Van Acker, 2008). There is no information to indicate change in survival capacity (including over-wintering).

The EFSA GMO Panel could not complete the assessment of the agronomic and phenotypic characteristics of cotton MON 15985 on the basis of the data provided (a single season and fewer than eight sites (EFSA, 2006a; EFSA GMO Panel 2011a)). Therefore, the EFSA GMO Panel could not conclude on the potential occurrence of unintended effects based on the outcome of the agronomic and

⁶⁹ Additional information: 12/03/2013.

⁷⁰ Additional information: 11/11/2013.

phenotypic analysis. The EFSA GMO Panel concludes that, considering the scope of this application, the aforementioned weight of evidence approach and the poor ability of cotton to survive outside cultivated land, there is very low likelihood that cotton MON 15985 has any enhanced fitness characteristics that will change its persistence and survival following accidental release into the environment of viable seeds from cotton MON 15985, except under conditions of infestation by the specific lepidopteran pests.

6.1.2.2. Potential for gene transfer⁷¹

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

(a) Plant to bacteria gene transfer

The recombinant DNA inserts in cotton MON 15985 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, 2009b). 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 a capacity to develop genetic competence for transformation (to take up extracellular DNA); (3) the mechanism of genetic recombination by which the plant DNA can be incorporated and thus 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).

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

Hazard identification and characterisation

Cotton MON 15985 contains recombinant genes and regulatory DNA sequences originating from bacteria, i.e. *aadA*, *nptII*, *oriV*, *uidA* and the *nos* promoter (see Section 3.1.4). 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 and a synthetic *cry2Ab2* gene encoding for a Cry2Ab variant protein of a *B. thuringiensis* strain. The *uidA*, *cry1Ac* and *cry2Ab2* genes are under the control of a promoter originating from the *Cauliflower mosaic virus* (CaMV) with the duplicated enhancer region (e35S). The *nptII* gene is under the control of the CaMV 35S promoter, while the *aadA* gene is under the control of its own promoter. The transcription of the aforementioned genes is under the control of the 3' untranslated region of the *nos* gene from *A. tumefaciens*, except the *cry1Ac* gene that is terminated by the soybean 7S 3' transcriptional termination sequence (for further details, see Section 3.1.3). The *cry1Ac* and *cry2Ab2* genes originate from *B. thuringiensis*, and in cotton MON 15985 they are under the control of an enhanced CaMV

⁷¹ Technical dossier, Section D6.

promoter mentioned above. The activity of the CaMV promoters in unrelated organisms such as bacteria cannot be excluded.

As described in Section 3.1.1, and as in the study performed within the frame of risk assessment for HGT of cotton MON 531 (EFSA, 2011b), bioinformatic analysis indicates the possibility of double homologous recombination between the *aadA* gene and the *oriV* present in cotton MON 15985 with the same sequences present in bacterial plasmids isolated from soil and activated sludge. This homologous recombination would lead to the replacement of the genes in such plasmids between the two recombination sites by the *nptII* gene cassette as present in the DNA of cotton MON 15985 and, thus, the acquisition of novel genetic information. The stabilisation rate of the *nptII* gene cassette in such bacteria is estimated from laboratory experiments with comparable constructs to be increased about 10^9 – 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. *aadA*, *uidA*, *nptII*, *cry1Ac* or *cry2Ab2*. Such substitutive recombination, however, would not lead to the acquisition of additional novel trait, since only nucleotide substitutions with existing genes would be expected. The potential for such replacements should be considered in the context of naturally occurring homologous recombination, mutations and additions or deletions in the bacterial genomes. Therefore, no hazard was identified.

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, 2009b) and are therefore not considered to contribute significantly to the HGT process.

Expression of the *nptII* gene under the control of CaMV 35S promoter has been demonstrated in bacteria (Assaad and Signer, 1990; Lewin et al., 1998). Therefore, oral treatment with kanamycin or neomycin may create a selective advantage for the transformed bacterial cells with the capability to express the *nptII*-encoded neomycin phosphotransferase II and could enhance further spread of *nptII* 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, 2009b).

This hazard identification and characterisation indicates that HGT of the *nptII* gene cassette of cotton MON 15985 could lead to kanamycin- and neomycin-resistant bacteria emerging in some environments, especially in the gastrointestinal tract or faeces of humans and animals receiving diets containing DNA of MON 15985, under selective conditions (i.e. usage of the corresponding antibiotics).

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 these applications (cotton MON 15985 for food and feed uses, import and processing, food additives produced from cotton MON 15985, feed produced from cotton MON 15985 (feed materials and feed additives); see Terms of reference), products that are covered in this application include seeds for feed use, oil for food and feed, meals, cake and hulls for feed, and linters and derived products (e.g. viscose, food casings, cellulose esters and ethers) for food. Based on the information provided by the applicant and knowledge from the literature it can be expected that recombinant DNA is still present in cottonseeds, cottonseed meal and linters. However, DNA was not

detected in methylcellulose or oil⁷². Experimental evidence was provided that processing reduced the content of transgenic DNA spanning the *nptII* gene cassette in the cottonseed meal from 1.6 to 5.2 % of what is present in unprocessed cottonseed⁷³.

In 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 cottonseeds and unprocessed linters because they may contain intact DNA. Exposure is also possible for products in which the transgenic DNA is more degraded but in which DNA of gene length size could still be present. For instance, such DNA is expected to be present in only limited quantities in cottonseed meal owing to the effects of processing. No exposure is expected from highly processed and refined products, such as cottonseed oil and methylcellulose, which covers all products of cotton MON 15985 relevant for human consumption. In animal feeding, cotton products are used in only small amounts in the EU (FEDIOL, online), mainly because of the presence of gossypol, which is highly toxic to non-ruminants (Verstraete, 2013)⁷⁴. Even with accepted upper limits of 500 mg/kg gossypol in feed for ruminants⁷⁵, the feed source will contain only a small percentage of cotton seeds or cottonseed meal. Because of the restricted dietary amounts, effects of feed processing and degradation in the gastrointestinal tract and faeces, the manure of animals fed with cotton MON 15985 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 15985 through manure or accidentally by decomposing seeds and decomposing plant material of occasional feral GM cotton plants originating from accidental cottonseed spillage during transportation or processing. 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, 2009b; 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 (Nordgård et al., 2007; EFSA, 2009b; 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 (Mercer et al., 1999a, b, 2001; Duggan et al., 2000, 2003).

Risk characterisation

Gastrointestinal bacteria of humans and animals and, in particular, of ruminants are expected to be exposed to the *aadA-nptII-oriV* DNA fragment from cotton MON 15985 by consumption of linters (consumed by humans and animals), cotton seeds and cottonseed meal (consumed by animals). Cotton seeds contain intact DNA, whereas cottonseed meal contains mainly fragmented DNA with a size smaller than that of the above-mentioned fragments⁷⁶. DNA is substantially 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 plant products are fed to animals in only low amounts in the EU (FEDIOL, online; Verstraete, 2013), the per animal exposure will be very low.

⁷² Additional information: 02/12/2010.

⁷³ Additional information: 02/12/2010.

⁷⁴ 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, p. 10–22.

⁷⁵ 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, p. 10–22.

⁷⁶ Additional information: 02/12/2010.

The *aadA* and *oriV* sequences that flank the *nptII* gene in cotton MON 15985 are present in naturally occurring bacteria in an arrangement which would allow double homologous recombination. The theoretical probability of horizontal transfer of the transgene sequences into bacteria is therefore higher compared to plant transgenes that do not have such flanking DNA sequences. The genetic composition of the inserted DNA in cotton MON 15985 facilitates homologous recombination with bacteria harbouring *aadA* and *oriV* sites in their DNA. Since such recombination sites are found located on mobile genetic elements, rare transfer of *nptII* 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 *nptII*-encoded resistance trait in environmental bacterial populations.

The contribution of HGT of the recombinant *nptII* 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 *nptII* gene must likewise be regarded relative to the natural distribution and prevalence of *nptII* genes on mobile genetic elements in bacteria. Bacteria carrying *nptII* on mobile genetic elements are found in various environments, although with large spatial and temporal fluctuations (EFSA, 2009b). Moreover, resistance genes other than *nptII* also lead to the distribution and prevalence of kanamycin- and neomycin-resistant bacteria in various environments.

There is limited information about the spatial and temporal variability in the selective conditions which would favour antibiotic-resistant bacteria, and in the occurrence, transferability and distribution of *nptII* genes in different environments. In addition, there is a lack of experimental data on HGT from cotton MON 15985.

Conclusion

The ERA indicates a negligible risk arising from a HGT of the *aadA*, *uidA*, *cry1Ac* and *cry2Ab2* genes from cotton MON 15985 to bacteria because of the highly limited potential for transfer. However, for products from cotton MON 15985 containing transgenic DNA, there is an increased likelihood of stabilisation of the *nptII* gene from plant DNA in bacteria compared with plants not including sites for double homologous recombination. This increased likelihood of transfer must, however, be seen in the context of the gene transfer efficiencies between bacteria, which remains several orders of magnitude higher.

Low-level exposure is expected for bacteria present in the gastrointestinal tracts of humans and animals. Considering the low level of DNA exposure per animal and, hence, the low frequency of gene transfer from MON 15985 to bacteria compared with gene transfer frequencies between bacteria, the GMO Panel concludes that MON 15985 material is highly unlikely to contribute to the environmental prevalence of *nptII* genes. In summary, the analysis of HGT from cotton MON 15985 to bacteria does not indicate a risk to human or animal health or to the environment in the context of its intended uses.

(b) Plant to plant gene transfer

Considering the intended uses of cotton MON 15985 and the physical characteristics of cotton seeds, a possible pathway of dispersal is from cottonseed spillage and pollen of occasional feral GM cotton plants originating from accidental cottonseed spillage during transportation and/or processing.

The genus *Gossypium* consists of at least four species: *G. arboreum*, *G. barbadense*, *G. herbaceum* and *G. hirsutum*. *G. herbaceum* is reported (Zohary and Hopf, 2000) to be a traditional fibre crop in the Eastern Mediterranean area already in the pre-Columbus period (before 1500 AD). In Southern Europe, *G. herbaceum* and *G. hirsutum* have been grown since the 19th century, giving rise to occasional feral plants in the same area (Davis, 1967; Tutin et al., 1992), but no sexually compatible wild relatives of *G. hirsutum* have been reported in Europe. Therefore, the plant to plant gene transfer from this GM cotton is restricted to cultivated and occasional feral populations.

Insect resistance to certain lepidopteran pests, such as CBW, PBW and TBW, provides an advantage in cultivation under infestation conditions. Survival of cotton outside cultivation in Europe is mainly limited by a combination of low competitiveness, absence of a dormancy phase, susceptibility to diseases and to cold climate conditions. Since these general characteristics of this GM cotton are unchanged, the inserted traits are not likely to provide a selective advantage outside cultivation in Europe (see Section 6.1.2.1).

The EFSA GMO Panel also takes into account the fact that this application does not include cultivation of the GM cotton MON 15985 within the EU so that the likelihood of cross-pollination between the imported GM cotton MON 15985 and cotton crops and occasional feral cotton plants is considered to be extremely low. Even if feral populations of cotton MON 15985 were established or transgene flow occurred to cultivated and feral cotton, a selective advantage would occur only under infestation of sensitive pest species.

6.1.2.3. Interactions of the GM plant with target organisms⁷⁷

Owing to the intended uses of cotton MON 15985, which exclude cultivation, and the low level of exposure to the environment, potential interactions of the GM plant with target organisms were not considered an issue by the EFSA GMO Panel.

6.1.2.4. Interactions of the GM plant with non-target organisms⁷⁸

Owing to the intended uses of cotton MON 15985, which excludes cultivation, and because of the low level of exposure to the environment, potential interactions of the GM cotton with non-target organisms (NTOs) were not considered an issue by the EFSA GMO Panel.

However, the EFSA GMO Panel evaluated whether the Cry proteins might potentially affect NTOs by entering the environment through faecal material from animals fed with this GM cotton. Owing to the specific insecticidal selectivity of Cry proteins, NTOs most likely to be affected by the Cry2Ab2 and Cry1Ac proteins belong to the same or closely related taxonomic group as those of the target organisms.

Data supplied by the applicant suggest that only low amounts of the Cry2Ab2 and Cry1Ac proteins enter the environment owing to low expression in cotton seeds (2.21–4.84 and 31.8–50.7 µg/g dry weight). Moreover, these Cry proteins are degraded by enzymatic activity in the gastrointestinal tract of animals fed on cotton MON 15985 or derived products (see Section 5.1.4.2), meaning that only low amounts of Cry proteins would remain intact to pass into faeces. This was demonstrated for Cry1Ab (Einspanier et al., 2004; Lutz et al., 2005, 2006; Wiedemann et al., 2006; Guertler et al., 2008). There would subsequently be further degradation of these Cry proteins in the faecal material due to intrinsic microbial proteolytic activity. In addition, there will be further degradation of Cry proteins in soil, reducing the possibility for the exposure of potentially sensitive NTOs. While Cry proteins may bind to clay minerals and humic substances in soil, thereby reducing their availability to microorganisms for degradation, there are no indications of persistence and accumulation of Cry proteins from GM crops in soil (reviewed by Icoz and Stotzky, 2008). The EFSA GMO Panel is not aware of evidence of released Cry proteins from GM plants causing significant negative effects on soil micro- or macroorganisms. Considering the scope of the application, it can be concluded that the exposure of potentially sensitive NTOs to the Cry2Ab2 and Cry1Ac proteins is likely to be very low and of no biological relevance.

⁷⁷ Technical dossier, Sections D8 and D9.4.

⁷⁸ Technical dossier, Section D9.5.

6.1.2.5. Potential interactions with the abiotic environment and biochemical cycles⁷⁹

Given the scope of this application, which excludes cultivation of cotton MON 15985, and the low level of exposure to the environment, potential interactions with the abiotic environment and biogeochemical cycles are not considered to be a relevant issue by the EFSA GMO Panel.

6.1.3. Post-market environmental monitoring⁸⁰

The objectives of a monitoring plan, according to Annex VII of Directive 2001/18/EC, are (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the ERA are correct and (2) to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment which were not anticipated in the ERA.

Monitoring is related to risk management and, thus, a final adoption of the PMEM plan falls outside the mandate of EFSA. However, the EFSA GMO Panel gives its opinion on the scientific content of the PMEM provided by the applicant (EFSA, 2006d; EFSA GMO Panel, 2011d). The potential exposure to the environment, including humans and animals, of cotton MON 15985 would be mainly ingestion by animals and their faecal material leading to exposure of gastrointestinal tract and soil microorganisms, and with the accidental release into the environment of viable cotton MON 15985 seeds during transport and/or processing.

The scope of the PMEM provided by the applicant is in line with the intended uses. As the ERA did not identify potential adverse environmental effects due to cotton MON 15985, no case-specific monitoring is required.

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

The EFSA GMO Panel is of the opinion that the scope of the PMEM proposed by the applicant is in line with the intended uses of cotton MON 15985 as the ERA did not cover cultivation and identified no potential adverse environmental effects. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

The EFSA GMO Panel advises that appropriate management systems should be in place to restrict seeds of cotton MON 15985 entering cultivation as the latter requires specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

6.2. Conclusion

The scope of applications EFSA-GMO-UK-2008-57 and EFSA-RX-MON15985 covers cotton MON 15985 for food and feed uses, import and processing, food additives produced from cotton MON 15985, feed produced from cotton MON 15985 (feed materials and feed additives) and does not include cultivation. Considering the intended uses of cotton MON 15985, the ERA is concerned with the exposure mainly through ingestion by animals and their faecal material leading to exposure of gastrointestinal tract and soil microorganisms, and with the accidental release into the environment of viable seeds of cotton MON 15985 during transport and processing.

In the case of accidental release into the environment of viable seeds of cotton MON 15985, there are no indications of an increased likelihood of establishment and spread of feral cotton MON 15985

⁷⁹ Technical dossier, Section D10.

⁸⁰ Technical dossier, Section D11.

plants, except under conditions of infestation of specific target pests. The low levels of environmental exposure of these GM cotton plants indicate that the risk to NTOs is extremely low.

No risk arising from the HGT of the *aadA*, *cry1Ac*, *cry2Ab2* and *uidA* genes from cotton MON 15985 to bacteria has been identified. An increased likelihood of stabilisation of the *nptII* gene from cotton MON 15985 DNA in bacteria was postulated. However, considering the expected low frequency of gene transfer from plants to bacteria compared with that between bacteria, and the low exposure to MON 15985 DNA, the GMO Panel concludes that it is highly unlikely that MON 15985 will contribute to the environmental prevalence of *nptII* genes. The analysis of HGT from cotton MON 15985 to bacteria does not indicate a risk to human or animal health or to the environment in the context of its intended uses.

The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of cotton MON 15985 and the guidance document of the EFSA GMO Panel on PMEM of GM plants (EFSA, 2006d; EFSA GMO Panel, 2011d). In addition, the EFSA GMO Panel acknowledges the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure due to possible cases of accidental release of viable seeds of cotton MON 15985. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the PMEM plan.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The EFSA GMO Panel was asked to carry out a scientific assessment of cotton MON 15985 for food and feed uses, import and processing in accordance with Regulation (EC) No 1829/2003.

The molecular characterisation data provided for cotton MON 15985 did not give rise to safety issues.

The EFSA GMO Panel could not complete the assessment of the agronomic and phenotypic characteristics of cotton MON 15985 on the basis of the data provided, derived from a single season and fewer than eight sites (EFSA, 2006a; EFSA GMO Panel 2011a). Therefore, the EFSA GMO Panel could not conclude on the potential occurrence of unintended effects based on the outcome of the agronomic and phenotypic analysis. The EFSA GMO Panel concludes that the compositional data give no indication that the genetic modification induces unintended effects for which further assessment is needed. The EFSA GMO Panel concludes that cotton MON 15985 is as safe and nutritious as its conventional counterpart and that it is unlikely that the overall allergenicity of the whole plant is changed.

Considering the intended uses of cotton MON 15985, the environmental risk assessment is concerned with the exposure through faecal material from animals fed with cotton products from cotton MON 15985 and with the accidental release into the environment of viable grains of cotton MON 15985 during transport and processing. Notwithstanding the incompleteness of the agronomic and phenotypic dataset, the EFSA GMO Panel followed a weight of evidence approach and, considering the scope of this application and the poor ability of cotton to survive outside cultivated fields, concluded that there is very low likelihood of any adverse environmental impacts due to the accidental release into the environment of viable seeds from cotton MON 15985. No risk arising from a HGT of the *aadA*, *cry1Ac*, *cry2Ab2* and *uidA* genes from cotton MON 15985 to bacteria has been identified. An increased likelihood of stabilisation of the *nptII* gene from cotton MON 15985 DNA in bacteria was postulated. However, considering the expected low frequency of gene transfer from plants to bacteria compared with that between bacteria, and the low exposure to MON 15985 DNA, the GMO Panel concludes that it is highly unlikely that MON 15985 will contribute to the environmental prevalence of *nptII* genes. The analysis of HGT from cotton MON 15985 to bacteria does not indicate a risk to human or animal health or to the environment in the context of its intended uses.

The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of cotton MON 15985 and the guidance document of the EFSA GMO Panel on PMEM of GM plants (EFSA 2006d; EFSA GMO Panel 2011d). In addition, the EFSA GMO Panel acknowledges the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure in cases of accidental release of viable seeds of cotton MON 15985. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the PMEM plan.

The EFSA GMO Panel considers that the dossiers presented by the applicant had deficiency in the data set relative to agronomic and phenotypic trials, however the EFSA GMO Panel concludes that cotton MON 15985, as described in applications EFSA-GMO-UK-2008-57 and EFSA-GMO-RX-MON15985, is as safe as its conventional counterpart and non-GM cotton commercial varieties and is unlikely to have adverse effects on human and animal health and the environment in the context of the scope of these applications.

DOCUMENTATION PROVIDED TO EFSA IN RELATION TO EFSA-GMO-UK-2008-57

1. Letter from the Competent Authority of the United Kingdom, received 22 May 2008, concerning a request for placing on the market of cotton MON 15985 × MON 1445 in accordance with Regulation (EC) No 1829/2003.
2. Acknowledgement letter, dated 5 June 2008, from EFSA to the Competent Authority of the United Kingdom.
3. Letter from EFSA to applicant, dated 17 July 2008, requesting additional information under completeness check
4. Letter from applicant to EFSA, received 24 July 2008, providing additional information under completeness check.
5. Letter from EFSA to applicant, dated 20 August 2008, delivering the ‘Statement of Validity’ of application EFSA-GMO-UK-2008-57 (cotton MON 15985 × MON 1445) submitted by Monsanto under Regulation (EC) No 1829/2003.
6. Letter from EFSA to applicant, dated 20 August 2008, requesting additional information and stopping the clock.
7. Letter from applicant to EFSA, received 2 September 2008, providing additional information.
8. Letter from EFSA to applicant, dated 24 November 2008, requesting additional information and maintaining the clock stopped.
9. Letter from EFSA to applicant, dated 7 April 2009, requesting additional information and maintaining the clock stopped.
10. Letter from EFSA to applicant, dated 28 May 2009, requesting additional information and maintaining the clock stopped.
11. Letter from EFSA to applicant, dated 18 September 2009, requesting additional information and maintaining the clock stopped.
12. Letter from EFSA to applicant, dated 15 March 2010, requesting additional information and maintaining the clock stopped.
13. Letter from applicant to EFSA, received 8 June 2010, providing additional information.
14. Letter from EFSA to applicant, dated 3 August 2010, requesting clarifications.
15. Letter from applicant to EFSA, received 15 September 2010, providing the clarifications requested.
16. Letter from EFSA to applicant, dated 4 October 2010, requesting additional information and maintaining the clock stopped.
17. Letter from applicant to EFSA, received 2 December 2010, providing additional information.
18. Letter from EFSA to applicant, dated 31 January 2011, requesting additional information and maintaining the clock stopped.
19. Letter from applicant to EFSA, received 11 April 2011, providing additional information.

20. Letter from EFSA to applicant, dated 21 September 2011, re-starting the clock.
21. Letter from EFSA to applicant, dated 5 December 2011, requesting additional information and stopping the clock.
22. Letter from EFSA to applicant, dated 6 July 2012, requesting additional information and maintaining the clock stopped.
23. Letter from EFSA to applicant, dated 12 July 2012, requesting clarifications on the progress of the application.
24. Letter from applicant to EFSA, received on 20 August 2012, providing clarifications on the progress of the applications.
25. Letter from applicant to EFSA, received 15 September 2012, providing clarifications on the progress of the application.
26. Letter from applicant to EFSA, received 14 September 2012, providing additional information.
27. Letter from EFSA to applicant, dated 9 October 2012, regarding the progress of the application.
28. Letter from applicant to EFSA, received 5 November 2012, providing additional information.
29. Letter from EFSA to applicant, dated 9 January 2013, requesting additional information and maintaining the clock stopped.
30. Letter from applicant to EFSA, received 12 March 2013, requesting clarifications on the EFSA letter dated 9 January 2013.
31. Letter from EFSA to applicant, dated 3 June 2013, requesting additional information and maintaining the clock stopped.
32. Letter from EFSA to applicant, dated 23 August 2013, requesting additional information and maintaining the clock stopped.
33. Letter from applicant to EFSA, received 5 November 2013, providing additional information.
34. Letter from applicant to EFSA, received 11 November 2013, providing additional information.
35. Letter from EFSA to applicant, dated 29 April 2014, re-starting the clock.

DOCUMENTATION PROVIDED TO EFSA IN RELATION TO EFSA-GMO-RX-MON15985

1. Letter from the European Commission, received 28 June 2007, concerning a request for renewal of the authorisation for the placing on the market of cotton MON 15985 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 3 December 2007, 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 18 March 2008, delivering the ‘Statement of Validity’ for application EFSA-GMO-RX-MON15985 (cotton MON 15985) submitted by Monsanto under Regulation (EC) No 1829/2003.
6. Letter from EFSA to applicant, dated 26 May 2008, requesting additional information and stopping the clock.
7. Letter from applicant to EFSA, received 28 October 2008, providing additional information.
8. Letter from EFSA to applicant, dated 24 November 2008, requesting additional information and maintaining the clock stopped.
9. Letter from applicant to EFSA, received 9 March 2009, providing additional information.
10. Letter from EFSA to applicant, dated 7 April 2009, requesting additional information and maintaining the clock stopped.
11. Letter from applicant to EFSA, received 18 May 2009, providing additional information.
12. Letter from EFSA to applicant, dated 26 May 2009, requesting additional information and maintaining the clock stopped.
13. Letter from EFSA to applicant, dated 18 September 2009, requesting additional information and maintaining the clock stopped.
14. Letter from applicant to EFSA, received 18 January 2010, providing additional information.
15. Letter from EFSA to applicant, dated 12 March 2010, requesting additional information and maintaining the clock stopped.
16. Letter from EFSA to applicant, dated 2 August 2010, requesting clarifications.
17. Letter from applicant to EFSA, received 15 September 2008, providing the clarifications requested.
18. Letter from applicant to EFSA, received 29 September 2008, providing clarifications.
19. Letter from EFSA to applicant, dated 4 October 2010, requesting additional information and maintaining the clock stopped.
20. Letter from applicant to EFSA, received 2 December 2010, providing additional information.
21. Letter from EFSA to applicant, dated 31 January 2011, requesting additional information and maintaining the clock stopped.
22. Letter from applicant to EFSA, received 11 April 2011, providing additional information.
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35. Letter from applicant to EFSA, received 5 November 2013, providing additional information.
36. Letter from applicant to EFSA, received 11 November 2013, providing additional information.
37. Letter from EFSA to applicant, dated 29 April 2014, re-starting the clock.

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