SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 2 March 2017

doi: 10.2903/j.efsa.2017.4746

Scientific opinion on an application by Monsanto (EFSA-GMO-NL-2013-114) for the placing on the market of a herbicide-tolerant genetically modified cotton MON 88701 for food and feed uses, import and processing under Regulation (EC) No 1829/2003

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Abstract

Cotton MON 88701 was developed through *Agrobacterium tumefaciens*-mediated transformation to express the dicamba mono-oxygenase (DMO) protein, conferring tolerance to dicamba, and the phosphinothricin *N*-acetyltransferase PAT protein, conferring tolerance to glufosinate ammonium-based herbicides. The molecular characterisation data and bioinformatics analyses did not identify issues requiring further assessment for food/feed safety. The agronomic and phenotypic characteristics tested revealed no relevant differences between cotton MON 88701 and its conventional counterpart. Since complete compositional results were reported for only three sites, the EFSA Panel on Genetically Modified Organisms (GMO Panel) is not in the position to complete the assessment of the compositional analysis. Moreover, as no 28-day toxicity study in rodents on the MON 88701 DMO protein was provided, the GMO Panel is not in the position to complete the safety assessment of this protein in cotton MON 88701. Consequently, the GMO Panel cannot complete the toxicological, allergenicity and nutritional assessment of food/feed derived from cotton MON 88701. The safety assessment identified no concerns regarding the potential toxicity and allergenicity of the PAT protein newly expressed in cotton MON 88701. Considering the routes of exposure and limited exposure levels, the GMO Panel concludes that cotton MON 88701 would not give rise to safety concerns in the event of accidental release of viable seeds into the environment. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of cotton MON 88701. In conclusion, in the absence of an appropriate comparative assessment and an appropriate assessment of the MON 88701 DMO protein, the GMO Panel is not in a position to complete its food/feed risk assessment of cotton MON 88701. The GMO Panel concludes that the cotton MON 88701 is unlikely to have any adverse effect on the environment in the context of the scope of the application.

Keywords: GMO, cotton (*Gossypium hirsutum*), MON 88701, herbicide tolerance, DMO, PAT, Regulation (EC) No 1829/2003

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Question number: EFSA-Q-2013-00219

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Competing interests: In line with EFSA's policy on declarations of interest, Panel member Philippe Guerche did not participate in the development and adoption of this scientific opinion.

Acknowledgements: The Panel wishes to thank the following for the support provided to this scientific opinion: the members of the Working group on Molecular Characterisation, Food and Feed Safety Assessment and Environmental Risk Assessment for the preparatory work on this scientific opinion.

Suggested citation: GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Birch AN, Casacuberta J, De Schrijver A, Gralak MA, Jones H, Manachini B, Messéan A, Nielsen EE, Nogué F, Robaglia C, Rostoks N, Sweet J, Tebbe C, Visioli F, Wal J-M, Poeting A, Álvarez F, Broll H and Ramon M, 2017. Scientific opinion on an application by Monsanto (EFSA-GMO-NL-2013-114) for the placing on the market of a herbicide-tolerant genetically modified cotton MON 88701 for food and feed uses, import and processing under Regulation (EC) No 1829/2003. EFSA Journal 2017;15(3):4746, 20 pp. doi: 10.2903/j.efsa.2017.4746

ISSN: 1831-4732

Summary

Following the submission of an application (EFSA-GMO-NL-2013-114) under Regulation (EC) No 1829/2003 by Monsanto, the Panel on Genetically Modified Organisms of the European Food Safety Authority (GMO Panel) was asked to deliver a scientific opinion on the safety of the herbicide-tolerant genetically modified (GM) cotton (*Gossypium hirsutum*) MON 88701 (Unique Identifier MON-887Ø1-3). The scope of application EFSA-GMO-NL-2013-114 is for import, processing, and food and feed uses of cotton MON 88701 within the European Union (EU), but excludes cultivation in the EU.

The GMO Panel evaluated cotton MON 88701 with reference to the scope and appropriate principles described in its guidelines for the risk assessment of GM plants. The evaluation addressed the following components of the risk assessment: the molecular characterisation of the inserted DNA and analysis of the expression of the corresponding protein; the comparative analyses of compositional, agronomic and phenotypic characteristics; the safety of the newly expressed proteins and the whole food/feed with respect to potential toxicity, allergenicity and nutritional characteristics; the environmental risk assessment and the post-market environmental monitoring (PMEM) plan.

Cotton MON 88701 was developed by *Agrobacterium tumefaciens*-mediated transformation of hypocotyl segments of dark grown cotton seedlings. It expresses the dicamba mono-oxygenase (DMO) and phosphinothricin *N*-acetyltransferase (PAT) proteins, which confers tolerance to dicamba- (3,6-dichloro-2-methoxybenzoic acid) and glufosinate ammonium-based herbicides, respectively. The molecular characterisation data established that cotton MON 88701 contains one functional insert consisting of intact DMO and PAT expression cassettes. No other parts of the plasmid used for transformation could be detected in cotton MON 88701. Bioinformatic analyses did not indicate significant similarities to toxins and allergens and genetic stability was demonstrated. The levels of the newly expressed protein present in cotton MON 88701 were obtained and reported adequately.

The comparative assessment of agronomic and phenotypic characteristics did not identify differences between cotton MON 88701 and its conventional counterpart requiring further assessment. The comparative assessment of compositional endpoints did not fulfil the minimum requirements described in the GMO Panel guidance for risk assessment of food and feed from GM plants (EFSA GMO Panel, 2011a). Complete compositional results were reported for only three sites. Each of the other sites lacked at least one replication of the conventional counterpart; in addition, two sites lacked also the required number of replications of the non-GM reference cotton varieties. Consequently, the GMO Panel is not in the position to complete the assessment of the compositional analysis.

The safety assessment identified no concerns regarding the potential toxicity and allergenicity of the PAT protein newly expressed in cotton MON 88701; however, no 28-day toxicity study in rodents on the MON 88701 DMO protein was provided. Therefore, the GMO Panel is not in the position to complete the safety assessment of this protein in cotton MON 88701. Since the composition of cotton MON 88701 cannot be assessed, the GMO Panel cannot complete the toxicological, allergenicity and nutritional assessment of food/feed derived from cotton MON 88701.

Considering the scope of this application, the environmental risk assessment is concerned with the accidental release into the environment of viable cotton MON 88701 seeds (i.e. during transport and/or processing), and with the exposure of bacteria to recombinant DNA in the gastrointestinal tract of animals fed GM material and those present in environments exposed to their faecal material (manure and faeces).

In case of accidental release into the environment of viable seeds of cotton MON 88701, there are no indications of an increased likelihood of establishment and spread of occasional feral cotton MON 88701 plants, unless these plants are exposed to the intended herbicides. However, this would not result in different environmental impacts compared to conventional cotton. Potential interactions with the biotic and abiotic environment are not considered to be an issue. Risks associated with an unlikely but theoretically possible horizontal gene transfer from cotton MON 88701 to bacteria have not been identified. The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of cotton MON 88701.

In delivering its scientific opinion, the GMO Panel took into account application EFSA-GMO-NL-2013-114, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications. In conclusion, in the absence of an appropriate comparative assessment and an appropriate assessment of the MON 88701 DMO protein, the GMO Panel is not in a position to complete its food/feed risk assessment of cotton MON 88701. However, the GMO Panel concludes that the cotton MON 88701 is unlikely to have any adverse effect on the environment in the context of the scope of this application.

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1. Introduction

Cotton MON 88701 was developed to confer tolerance to dicamba- and glufosinate ammonium-based herbicides. Tolerance to dicamba (3,6-dichloro-2-methoxybenzoic acid) is achieved by the expression of a *dmo* gene from *Stenotrophomonas maltophilia* encoding the dicamba mono-oxygenase (DMO) enzyme. Tolerance to glufosinate ammonium-based herbicides is achieved by the expression of a *bar* gene from *Streptomyces hygroscopicus* that encodes the phosphinothricin *N*-acetyltransferase (PAT) protein.[1]

The assessment of potential consumer health risks resulting from dicamba and its metabolites in cotton MON 88701 is outside the remit of the Panel on Genetically Modified Organisms of the European Food Safety Authority (GMO Panel) and needs to be performed upon request of an applicant in the framework of Regulation (EC) No 396/2005.

1.1. Background

On 13 February 2013, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands an application (Reference EFSA-GMO-NL-2013-114) for authorisation of genetically modified (GM) cotton MON 88701 (Unique Identifier MON-887Ø1-3), submitted by Monsanto within the framework of Regulation (EC) No 1829/2003 on GM food and feed.[2]

After receiving the application EFSA-GMO-NL-2013-114, and in accordance with Articles 5(2)(b) and 17(2)(b) of the Regulation (EC) No 1829/2003, EFSA informed the Member States and the European Commission, and made the summary of the application publicly available on the EFSA website.[3] EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of the Regulation (EC) No 1829/2003. On 24 April 2013 and 4 June 2013, EFSA received additional information requested under completeness check (on 27 March 2013 and 21 May 2013, respectively). On 25 June 2013, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the European Commission, and consulted nominated risk assessment bodies of Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC[4] following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Member States had 3 months after the date of receipt of the valid application (until 21 October 2013) to make their opinion known.

On 13 September 2013, 12 November 2013, 19 December 2013, 19 May 2014, 5 June 2014, 18 December 2014, 3 June 2015, 9 September 2015, 23 March 2016 and 12 December 2016, the GMO Panel requested additional information from the applicant. The applicant provided the requested information on 24 January 2014, 10 February 2014, 1 August 2014, 22 September 2014, 20 February 2015, 13 August 2015, 11 January 2016, 24 November 2016 and 20 January 2017. The applicant also requested clarifications on 7 October 2015 and 11 January 2016, provided by EFSA on 26 October 2015 and 12 February 2016. Furthermore, the applicant provided clarification on the additional information submitted on 18 February 2015 during a Food and Feed Safety Working Group meeting held on 3–4 May 2016.[5]

In the frame of contract OC/EFSA/UNIT/GMO/2013/01 and CFT/EFSA/AMU/2011/01, the contractors performed preparatory work and delivered reports on the information provided and the methods applied by the applicant in performing bioinformatic and statistical analyses, respectively.

In giving its scientific opinion on cotton MON 88701 to the European Commission, 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 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.

[1] Dossier: Part II – Section A.2.2.1.
[2] 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, 18.10.2003, p. 1–23.
[3] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2013-00219
[4] 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, 12.3.2001, p. 1–38.
[5] http://www.efsa.europa.eu/sites/default/files/gmofoodfeed_20082016.pdf (pages 77–78)

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 EFSA overall opinion in accordance with Articles 6(5) and 18(5).

1.2. Terms of Reference as provided by the requestor

The GMO Panel was requested to carry out a scientific assessment of cotton MON 88701 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003.

Where applicable, any conditions or restrictions which should be imposed on the placing on the market and/or specific conditions or restrictions for use and handling, including post-market monitoring requirements based on the outcome of the risk assessment and, in the case of genetically modified organisms (GMOs) or food/feed containing or consisting of GMOs, conditions for the protection of particular ecosystems/environment and/or geographical areas should be indicated in accordance with Articles 6(5)(e) and 18(5)(e) of Regulation (EC) No 1829/2003.

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

2. Data and methodologies

2.1. Data

In delivering its scientific opinion, the GMO Panel took into account application EFSA-GMO-NL-2013-114, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications.

2.2. Methodologies

The GMO Panel carried out an evaluation of the scientific risk assessment of cotton MON 88701 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. The GMO Panel took into account the appropriate principles described in its guidelines for the risk assessment of GM plants and derived food and feed (EFSA GMO Panel, 2011a), the environmental risk assessment (ERA) of GM plants (EFSA GMO Panel, 2010b) and on the post-market environmental monitoring (PMEM) of GM plants (EFSA GMO Panel, 2011b).

The comments raised by Member States are addressed in Annex G of the EFSA overall opinion[3] and were taken into consideration during the evaluation of the risk assessment.

3. Assessment

3.1. Molecular characterisation

3.1.1. Evaluation of relevant scientific data

3.1.1.1. Transformation process and vector constructs[6]

Cotton MON 88701 was developed by *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*)-mediated transformation of hypocotyl segments of dark grown cotton seedlings (*Gossypium hirsutum*) cv. Coker 130 with plasmid vector PV-GHHT6997.

The plasmid PV-GHHT6997 used for the transformation contained two expression cassettes between the right and left borders of the T-DNA: *dmo* and *bar*.

The *dmo* expression cassette contains the following genetic elements: the PC1SV promoter from the *Peanut chlorotic streak caulimovirus*; the 5' leader sequence from the *Tobacco etch virus* (TEV); the sequence encoding CTP2 from the *Arabidopsis thaliana shkG* gene; *dmo*, the codon-optimised coding sequence of the DMO protein from *S. maltophilia*; the 3' non-translated sequence of the E6 gene from *Gossypium barbadense*.

[6] Dossier: Part II – Sections A.2.1.2 and A.2.1.3.

The *bar* expression cassette contains the following genetic elements: a 611 bp e35S promoter, containing a duplicated enhancer region, from the *Cauliflower mosaic virus*; the 5′ non-translated leader sequence from the *Petunia hybrida DnaK* gene encoding heat shock protein 70 (Hsp70); *bar*, the coding sequence of the PAT protein from *S. hygroscopicus* and the 3′ untranslated region (UTR) sequence of the nopaline synthase (*nos*) gene from *A. tumefaciens*.

The vector backbone contained elements necessary for the maintenance of the plasmid in bacteria.

3.1.1.2. Transgene constructs in the GM plant[7]

Molecular characterisation of cotton MON 88701 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 sequences. The approach used was acceptable both in terms of coverage and sensitivity.

Southern analyses indicated that cotton MON 88701 contains a single insert, which consists of a single copy of the *dmo* and *bar* expression cassettes from the PV-GHHT6997 vector. The insert and copy number were confirmed by multiple restriction enzyme/probe combinations covering the T-DNA region and flanking regions. PCR analyses confirmed the results obtained by the Southern analyses. The absence of vector backbone sequences was demonstrated by Southern analysis using backbone-specific overlapping probes.

The nucleotide sequence of the entire insert of cotton MON 88701 together with 1,126 bp of the 5′ and 1,138 bp of the 3′ flanking regions were determined. The 4,105 bp insert is identical to the T-DNA of PV-GHHT6997, except for the deletion of the right border region and 9 bp of the adjacent intervening sequence and the deletion of 180 bp of the left border. A comparison with the pre-insertion locus indicated that 123 bp were deleted from the cotton genomic DNA. The possible interruption of known endogenous cotton genes by the insertion in event MON 88701 was evaluated by bioinformatic analyses of the pre-insertion site and of the genomic sequences flanking the insert. The results of these analyses did not indicate the interruption of any known endogenous gene in cotton MON 88701. However, there was evidence that the inserted DNA landed in a region of retrotransposon-like sequence. Approximately 65% of the cultivated cotton genome is made of retrotransposon-like sequences (Li et al., 2015). Most of these sequences are remnants of ancient retrotransposons and are no longer active. In addition, most of the retrotransposon-related individual sequences do not fulfil a specific role in the genome.[8]

The results of segregation (see Section 3.1.1.5) and bioinformatic analyses established that the insert is located in the nuclear genome.

Updated bioinformatic analyses of the amino acid sequence of the newly expressed DMO and PAT proteins revealed no significant similarities to toxins and allergens. In addition, updated bioinformatics analyses of the newly created open reading frames (ORFs) within the insert and spanning the junctions between the insert and genomic DNA did not indicate significant similarities to toxins and allergens.[8]

In order to assess the possibility for horizontal gene transfer (HGT) by homologous recombination (HR), the applicant performed a sequence identity analysis of the regions of bacterial origin in cotton MON 88701. The likelihood and potential consequences of plant-to-bacteria gene transfer are described in Section 3.4.1.1.

3.1.1.3. Protein characterisation and equivalence

Cotton MON 88701 expresses two new proteins, DMO and PAT. Given the technical restraints in producing large enough protein quantities for safety testing from plants, these proteins were recombinantly produced in *Escherichia coli*.

Prior to safety studies, a Panel of biochemical methods were employed to demonstrate the equivalence between cotton and microbe-produced proteins. Purified proteins from these sources were characterised and compared in terms of their physicochemical, structural and functional properties.[9]

DMO characterisation and equivalence

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis showed that full-length plant and microbe-produced DMO proteins had the expected molecular weight

[7] Dossier: Part II – Section A.2.2.2.
[8] Additional information received on 13/8/2015.
[9] Dossier: Part II – Section A.4.2 (Study reports: MSL 0023517 and MSL 0023428).

of ~ 39 kDa and were comparably immunoreactive to DMO protein specific polyclonal antibodies. Glycosylation detection analysis demonstrated that none of the DMO proteins were N-glycosylated. Amino acid sequence analysis by mass spectrometry and N-terminal sequencing[10] methods showed that both proteins matched their expected sequence. These data also showed that the N-terminus of both proteins contains an additional nine residues derived from the cleavage of the chloroplast transit peptide CTP2, included to target the DMO protein to the chloroplast. The N-terminus of both proteins also contains an additional alanine at position 2.[10] Furthermore, the N-terminal valine of the plant derived DMO protein was acetylated. Functional equivalence was demonstrated by a biochemical *in vitro* activity assay which showed that both proteins had comparable activity for the intended herbicide. Protein crystal soaking experiments in solutions of dicamba or *o*-anisic acid showed that DMO protein has a high specificity for dicamba. Microbial-produced DMO protein was also screened for its ability to utilise certain endogenous plant substrates and none of them were metabolised by DMO.[11]

PAT characterisation and equivalence

SDS-PAGE and western blot analysis showed that plant and microbe-produced PAT proteins had the expected molecular weight of ~ 21 kDa and were comparably immunoreactive to PAT protein specific polyclonal antibodies. Glycosylation detection analysis demonstrated that none of the PAT proteins were N-glycosylated. Amino acid sequence analysis by mass spectrometry and N-terminal sequencing methods showed that both proteins matched their expected sequence. These data also showed that fractions of the plant derived protein had its N- terminal methionine and serine residues truncated. Functional equivalence was demonstrated by a biochemical *in vitro* activity assay which showed that both proteins had comparable activity for the intended herbicide.

The protein characterisation data comparing the structural, biochemical and functional properties of plant and microbial produced DMO and PAT proteins, indicate that these proteins are equivalent.

3.1.1.4. Information on the expression of the insert[12]

Protein levels of DMO and PAT proteins were analysed by enzyme-linked immunosorbent assay (ELISA) in material harvested from replicated field trials across eight locations in the USA during the 2010 growing season. Samples analysed included leafs (OSL 1, 2, 3, 4), root, pollen and seed from those treated with dicamba and glufosinate, and seed from those not treated with the intended herbicide. The mean values and ranges of protein expression levels in seeds (n = 32) of the DMO and PAT proteins are summarised in Table 1.

Table 1: Protein expression data for the DMO and PAT proteins in cotton MON 88701 seeds (μg/g dry weight)

	Not treated with dicamba and glufosinate	Treated with dicamba and glufosinate
DMO	18[(a)] ± 4.1[(b)] (11–27)[(c)]	21 ± 5.0 (8.9–33)
PAT	6.3 ± 1.3 (4.0–9.1)	6.6 ± 1.1 (5.2–9.6)

DMO: dicamba mono-oxygenase; PAT: phosphinothricin *N*-acetyltransferase.
(a): Mean.
(b): Standard deviation.
(c): Range.

3.1.1.5. Inheritance and stability of inserted DNA[13]

Genetic stability of the cotton MON 88701 insert was assessed by Southern analysis of genomic DNA from five different generations (R2–R6). The restriction enzyme/probe combinations used were sufficient to conclude that all the plants tested retained the single copy of the insert and flanking regions, which were stably inherited in subsequent generations.

Phenotypic stability was observed by segregation analysis of the glufosinate tolerance trait of cotton MON 88701. The results supported the presence of a single insertion, segregating in a Mendelian fashion.

[10] Additional information received on 24/1/2014.
[11] Dossier: Part II – Section A.2.3 (Study report: RPN-10-365).
[12] Dossier: Part II – Section A.2.2.3.
[13] Dossier: Part II – Section A.2.2.4.

3.1.2. Conclusion on the molecular characterisation

The molecular characterisation data establish that cotton MON 88701 contains a single insert consisting of one copy of the *dmo* and *bar* expression cassettes. Bioinformatic analyses of the sequences encoding the newly expressed proteins and other ORFs within the insert or spanning the junctions between the insert and genomic DNA did not indicate significant similarities to toxins and allergens. The stability of the inserted DNA and of the introduced herbicide tolerance trait was confirmed over several generations. The levels of the DMO and PAT protein were obtained and reported adequately. The protein characterisation data comparing the structural, biochemical and functional properties of plant and microbial derived DMO and PAT proteins, indicate that these proteins are equivalent and the microbial produced proteins can be used in the safety studies.

3.2. Comparative analysis

3.2.1. Evaluation of relevant scientific data

3.2.1.1. Choice of comparator and production of material for the comparative analysis

Application EFSA-GMO-NL-2013-114 presents data on agronomic and phenotypic characteristics, as well as seed composition, of cotton MON 88701 derived from field trials performed at eight sites in the USA in 2010 (Table 2).

Table 2: Overview of comparative assessment studies with cotton MON 88701 provided in application EFSA-GMO-NL-2013-114

Study focus	Study details	Comparator	Non-GM reference varieties
Agronomic and phenotypic characteristics	Field trials, 2010, US (eight locations)(b)	Coker 130	Eight(a)
	Pollen characteristics study, controlled conditions	Coker 130	Four
	Seed germination study, controlled conditions	Coker 130	Four
Compositional analysis	Field trials, 2010, US (eight locations)(b)	Coker 130	Eight

GM: Genetically modified.
(a): Four different varieties were grown at each location.
(b): Agronomic, phenotypic and compositional data studies were obtained from the same field trial study.

Field trials were performed in major cotton growing regions of the USA,[14] representing regions of diverse agronomic practices and environmental conditions. At each site, the following materials were grown in a randomised complete block design with four replicates: cotton MON 88701 not treated with the intended herbicides (MON 88701/untreated), cotton MON 88701 treated with dicamba + glufosinate (MON 88701/dicamba + glufosinate), the non-GM comparator Coker 130 and four non-GM cotton reference varieties. All materials were treated (sprayed) with required maintenance pesticides according to local requirements. In total, eight non-GM cotton reference varieties were included across all the field trials sites.[15]

Pollen seed characteristics from cotton MON 88701, the non-GM comparator Coker 130, and four non-GM cotton reference varieties were evaluated under laboratory (growth chamber) conditions (Table 2).

Coker 130 was the cotton (*Gossypium hirsutum* L.) variety originally transformed to obtain cotton event MON 88701. Therefore, the GMO Panel considered Coker 130 to have a comparable genetic background to the GM cotton and to be a suitable conventional counterpart.

Statistical analysis of field trials data

The statistical analysis of agronomic and phenotypic data from the 2010 field trials followed the recommendations of the GMO Panel (EFSA GMO Panel, 2010a, 2011a). This includes, for each of the two treatments of cotton MON 88701, the application of a difference test (between the GM plant and

[14] The eight sites were: Desha County, Arkansas (ARTI); Tift County, Georgia (GACH); Pawnee County, Kansas (KSLA); Rapides County, Louisiana (LACH); Perquimans County, North Carolina (NCBD); Dona Ana County, New Mexico (NMLC); Barnwell County, South Carolina (SCEK) and Hale County, Texas (TXPL).
[15] The following non-GM cotton reference varieties were included: Atlas, Delta Opal, DP 435, DP 5415, DP 565, FM 989, NM 1517-99, SG 125, ST474.

its conventional counterpart) and an equivalence test (between the GM plant and the set of non-GM commercial reference varieties). The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).[16]

3.2.1.2. Agronomic and phenotypic characteristics

Agronomic and phenotypic characteristics tested under field conditions[17]

Twenty-six agronomic and phenotypic endpoints were analysed.[18] The following significant differences were found between MON 88701 and its conventional counterpart Coker 130:

- For MON 88701/untreated, significant differences were found for plant height at 30 days after planting (DAP), plant height at harvest, nodes above white flower (for all three observations), total number of bolls, total number of bolls at first position, percentage of boll retention at first position, seed index, number of seeds per boll, number of mature seed per boll, fibre micronaire and fibre strength. The test of equivalence showed that all these characteristics were equivalent to the non-GM cotton reference varieties (equivalence category I).
- For MON 88701/dicamba + glufosinate, significant differences were found for plant height at 30 DAP, plant height at harvest, nodes above white flower (for all three observations), number of nodes to first fruiting branch, total bolls, total number of bolls at first position, percentage of boll retention at first position, seed index, number of seeds per boll, number of mature seeds per boll, number of immature seeds per boll, fibre elongation and fibre strength. The test of equivalence showed that all these characteristics were equivalent to the non-GM cotton reference varieties (equivalence category I).

No altered stress responses of cotton MON 88701, compared with its conventional counterpart, with regard to visually observable response to naturally occurring diseases, abiotic stress and arthropod damage were observed.

Agronomic and phenotypic characteristics tested under controlled conditions[19]

1) Pollen characteristics

The applicant reported data on pollen characteristics of cotton MON 88701. The endpoints analysed were pollen diameter, and viability via the Alexander stain method. No significant difference between cotton MON 88701 and its conventional counterpart was observed for pollen diameter. Although Alexander's stain is intended to provide an indication of the viability, it does not directly measure pollen viability (Dafni, 1992). Therefore, data on pollen viability supplied by the applicant in support of the comparative assessment of cotton MON 88701 is not considered suitable by the GMO Panel. Given that the genetic modification of cotton MON 88701 is not designed to target specific pollen characteristics, the GMO Panel considers that data provided on pollen are not required for the risk assessment of cotton MON 88701.

2) Seed characteristics

The applicant reported data on seed characteristics of cotton MON 88701. Seed germination tests with F_2 seeds harvested from cotton MON 88701, its conventional counterpart and four non-GM cotton varieties, grown under field conditions at three field sites in 2010, were performed under growth chamber conditions. Event-specific PCR analyses in the conventional counterpart and in the reference varieties seed lots revealed presence of the transformation event MON 88701 in the non-GM lines (from $\leq$ 0.99% to $\leq$ 5.65% at two sites and from $\leq$ 0.99% to $\leq$ 3.05% at one site).[20] Given the low level of GM seeds detected in the control material level, the GMO Panel is of the opinion that the seed germination study can still be used to support the comparative analysis of cotton MON 88701.

[16] The results of the equivalence test are categorised into four possible outcomes are: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence) and category IV (indicating non-equivalence).
[17] Dossier: Part II – Section A.3.4.2. Additional information: 1/8/2014.
[18] Stand count (3 observations), plant height (at 30 days after planting and at harvest), number of nodes above white flower (3 observations), yield, main stem nodes, nodes to first fruiting branch, total bolls, total first position bolls, total vegetative bolls, percent retention first position bolls, percent first position bolls, seed index, total seed per boll, mature seed per boll, immature seed per boll, boll weight, fibre micronaire, fibre elongation, fibre strength, fibre length and fibre uniformity.
[19] Dossier: Part II – Sections A.3.4.4 and A.3.4.5.
[20] The values represent an estimated 95% upper confidence intervals.

Seeds were incubated under controlled conditions at six different temperature regimes. The endpoints analysed were the number of germinated normal seeds, germinated abnormal seeds, hard seeds, dead seeds and firm swollen seeds. No significant differences between cotton MON 88701 and its conventional counterpart were observed for any endpoint, except for % of germinated seeds and % of dead seeds at 30°C. The observed values in cotton MON 88701 for both endpoints were within the range of those observed in the reference varieties. The GMO Panel therefore concluded that observed differences were not relevant.

3.2.1.3. Compositional analysis

According to the guidance for risk assessment of food and feed from GM plants of the GMO Panel (EFSA GMO Panel, 2011a), each field trial should be replicated at a minimum of eight sites and the replication should never be less than four at any site. Compositional results fulfilling these requirements were reported for only three sites. Each of the other sites lacked at least one replication of the conventional counterpart; in addition, two sites lacked also the required number of replications of the non-GM reference cotton varieties. Although requested, the applicant did not provide additional field trials data for cotton MON 88701.[21] The GMO Panel is therefore not in the position to complete the assessment of the compositional analysis.

3.2.2. Conclusion on comparative analysis

The GMO Panel concludes that none of the observed differences in the agronomic and phenotypic characteristics between cotton MON 88701 and its conventional counterpart requires further assessment regarding environmental, food and feed safety.

Because of the lack of an appropriate dataset, the GMO Panel is not in the position to conclude on the compositional analysis and can therefore not complete the comparative analysis.

3.3. Food/feed safety assessment

3.3.1. Evaluation of relevant scientific data

3.3.1.1. Effects of processing

The thermal stability of the bacterial DMO protein (*E. coli* produced MON 88701 DMO protein) was evaluated by heating the protein in aqueous buffered solution for 15 and 30 min at 25, 37, 55, 75 and 95°C. At temperatures of 55°C and above for 15 and 30 min, a loss of functional activity below the limit of quantification (LOQ) (1.5 nmol DCSA $\times$ min^{-1} $\times$ mg^{-1}) was observed. SDS-PAGE analysis demonstrated that the molecular mass of the DMO protein (approximately 38 kDa) was unchanged at all tested heat treatments for 15 and 30 min since no effect on the band intensity of the DMO protein was observed.[22]

The thermal stability of the bacterial PAT protein (*E. coli* produced MON 88701 PAT protein) was evaluated by heating the protein in aqueous buffered solution for 15 and 30 min at 25, 37, 55, 75 and 95°C. At temperature of 55°C for 15 and 30 min a loss of functional activity of, respectively, 76% and 60% was observed, exceeding 90% at temperatures of 75 and 95°C for 15 and 30 min. SDS-PAGE analysis demonstrated that the molecular mass of the PAT protein (approximately 25 kDa) was unchanged at all tested heat treatments for 15 and 30 min since no effect on the band intensity of the DMO protein was observed.[23]

Because the GMO Panel is not in the position to conclude on the compositional analysis and therefore cannot complete the assessment of the comparative analysis, it is also not in the position to assess the effect of processing of cotton MON 88701.

3.3.1.2. Toxicology

Toxicological assessment of newly expressed proteins

The PAT protein has been previously assessed by the GMO Panel and no safety concerns for humans and animals were identified (e.g. EFSA GMO Panel, 2007). Updated bioinformatics analysis did not reveal similarities between this newly expressed protein and known toxins (Section 3.1.1.2). The

[21] Additional information received on 22/9/2014.
[22] Dossier: Part II – Section A.3.5 (Study report MSL0023606).
[23] Dossier: Part II – Section A.3.5 (Study report MSL0023584).

GMO Panel is not aware of any new information that would change these conclusions. The GMO Panel concludes that the PAT protein does not raise safety concerns.

The GMO Panel has previously assessed the safety of a DMO protein in soybean MON 87708, where it was present in two variants (i.e. DMO and DMO + 27). In that context, upon request of the GMO Panel to confirm the safety, a 28-day toxicity study in mice with the mixture of the two variants was provided (EFSA GMO Panel, 2013). The GMO Panel noted that the DMO protein present in cotton MON 88701 structurally differs from both variants present in soybean MON 87708, at the N-terminus and in the internal amino acid sequence (two amino acids), and functional equivalence was not demonstrated. As no 28-day toxicity study in rodents on the MON 88701, DMO protein was provided, following an EFSA request, the GMO Panel is not in the position to complete the toxicological assessment of this protein.

The applicant also provided *in vitro* degradation and acute toxicity studies on *E. coli* produced MON 88701 DMO and PAT proteins (Section 3.1.1.3).

a) *In vitro* degradation studies

The resistance to degradation by pepsin of the *E. coli* produced MON 88701 DMO and PAT proteins were investigated in solutions at pH ~ 1.2 in two independent studies. The integrity of the test proteins in samples taken at various time points were analysed by SDS-PAGE followed by protein staining or western blot. The DMO and the PAT proteins were degraded by pepsin within 30 s.

In addition, the applicant also performed a standalone degradation study of DMO and PAT proteins in so called simulated intestinal fluid (SIF) according to a method previously described (USP, 1995) in which SIF designates a mixture of proteolytic enzymes known as pancreatin. The GMO Panel notes that the resistance to degradation by standalone SIF is currently not specifically required by either the EFSA guidance document (EFSA GMO Panel, 2011a) or Codex Alimentarius (2009). Due to the intrinsic limitations of such standalone SIF degradation study for the food and feed safety of newly expressed proteins, it was not considered in the overall safety assessment.

b) Acute oral toxicity testing

The *E. coli* produced MON 88701 DMO protein was administrated by oral gavage at a dose of 283 mg/kg body weight (bw) to male and female Crl:CD1 mice. No adverse effects related to the DMO protein were observed.

The *E. coli* produced MON 88701 PAT proteins was administrated by oral gavage at a dose of 1,086 mg/kg bw to male and female Crl:CD1 mice. No adverse effects related to the PAT protein were observed.

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

Toxicological assessment of components other than newly expressed proteins

Because the GMO Panel is not in the position to conclude on the compositional analysis and therefore cannot complete the assessment of the comparative analysis, it is also not in the position to complete the assessment of components other than the newly expressed proteins.

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

No animal studies with food/feed derived from cotton MON 88701 were provided by the applicant.

Because the GMO Panel is not in the position to conclude on the compositional analysis and therefore cannot complete the assessment of the comparative analysis, it is also not in the position to assess the need for animal studies with the food/feed derived from this GM cotton.

3.3.1.4. 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.

Assessment of allergenicity of the newly expressed proteins[24]

A weight-of-evidence approach was followed, taking into account all of the information obtained on the newly expressed protein, as no single piece of information or experimental method yield sufficient evidence to predict allergenicity (Codex Alimentarius, 2009; EFSA GMO Panel 2011a).

The *dmo* gene (encoding for the DMO protein) and the *pat* gene (encoding for the PAT protein) originate from *S. maltophilia* and *S. hygroscopicus*, respectively, which are not considered to be allergenic sources.

Updated bioinformatic analyses[8] of the amino acid sequences of the DMO and PAT proteins, using the criterion of 35% identity in a sliding window of 80 amino acids, revealed no significant similarities to known allergens. In addition, the applicant also performed analyses searching for matches of eight contiguous identical amino acid sequences between the DMO and PAT proteins and known allergens, which confirmed the outcome of the previous bioinformatic analysis.

The studies on resistance to degradation of the DMO and PAT proteins by pepsin have been described in Section 3.3.1.2.

The GMO Panel has previously evaluated the safety of the PAT protein in the context of several applications and no concerns on allergenicity were identified (e.g. EFSA GMO Panel, 2007).

There is no information available on the structure or function of the newly expressed DMO and PAT proteins that would suggest an adjuvant effect of the proteins, individually or simultaneously, in cotton MON 88701 resulting in or enhancing an eventual specific immunoglobulin E (IgE) response to a bystander protein.

In the context of the present application and following current principles described by GMO Panel guidelines (EFSA GMO Panel, 2011a) and Codex Alimentarius (2009), the GMO Panel considers that there are no indications that the newly expressed DMO and/or PAT proteins in cotton MON 88701 may be allergenic. It is noted that, as no 28-day toxicity study in rodents on the MON 88701 DMO protein was provided, following an EFSA request, the GMO Panel is not in the position to complete the toxicological assessment of this protein, including that on the immune system.

Assessment of allergenicity of the whole GM plant or crop[25]

The GMO Panel regularly reviews the available publications on food allergy to cottonseed (EFSA GMO Panel, 2016). However, to date, cotton has not been considered to be a common allergenic food[26] (OECD, 2009). Therefore, the GMO Panel did not request experimental data to analyse the allergen repertoire of GM cotton and the assessment is performed based on considerations from the molecular characterisation, the compositional analysis and the assessment of the newly expressed proteins.

Since the composition of cotton MON 88701 cannot be assessed (Section 3.2.1.3) and in the light of its relevance for the identification of possible unintended effects, the GMO Panel cannot conclude on the allergenicity of the whole GM plant.

3.3.1.5. Nutritional assessment of GM food/feed

Because the GMO Panel is not in the position to conclude on the compositional analysis and therefore cannot complete the assessment of the comparative analysis, it is also not in the position to complete the nutritional assessment of GM food/feed.

3.3.1.6. Post-market monitoring of GM food/feed

Because the GMO Panel is not in the position to conclude on the compositional analysis and therefore cannot complete the assessment of the comparative analysis, it is also not in the position to comment on post-market monitoring of the GM food/feed.

3.3.2. Conclusion on the food/feed safety assessment

The safety assessment identified no concerns regarding the potential toxicity and allergenicity of the PAT protein newly expressed in cotton MON 88701. As no 28-day toxicity study in rodents on the MON 88701 DMO protein was provided, the GMO Panel is not in the position to complete the safety

[24] Dossier: Part II – Sections A5.1 and A5.3.
[25] Dossier: Part II – Section A5.2.
[26] 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.

assessment of this protein in cotton MON 88701. Because of the lack of an appropriate compositional dataset, the GMO Panel cannot complete the toxicological, allergenicity and nutritional assessment of food/feed derived from cotton MON 88701.

3.4. Environmental risk assessment and monitoring plan

3.4.1. Evaluation of relevant scientific data

Considering the scope of application EFSA-GMO-NL-2013-114, which excludes cultivation, the environmental risk assessment (ERA) of cotton MON 88701 is mainly concerned with: (1) the exposure of bacteria to recombinant DNA in the gastrointestinal tract of animals fed GM material and bacteria present in environments exposed to faecal material (manure and faeces); and (2) the accidental release into the environment of viable seeds of cotton MON 88701 during transportation and/or processing (EFSA GMO Panel, 2010b).

3.4.1.1. Environmental risk assessment

Persistence and invasiveness of the GM plant[27]

In southern Europe, *Gossypium herbaceum* and *G. hirsutum* have been grown since the 19th century and led to transient or locally naturalised cotton plants in the same area (Davis, 1967; Tutin et al., 1992; Sarno et al., 1993; Celesti-Grapow et al., 2010). However, survival of cottonseeds outside cultivation areas in Europe is limited due to the absence of a seed dormancy phase. Even if seeds from spillage germinate, the resulting cotton plants are unlikely to survive due to factors such as cold climatic conditions, the susceptibility to diseases and their low competitiveness (Eastick and Hearnden, 2006). For example, after the end of cotton cultivation in Italy in 1950s, no feral cotton was reported in southern Italy, except in some restricted areas (Sarno et al., 1993; Celesti-Grapow et al., 2010). Also, in other cotton-growing regions, such as in Australia, surveys showed that feral GM cotton established infrequently along transportation routes and mostly as transient populations (Addison et al., 2007).

The *dmo* and *pat* genes coding for the herbicide tolerance trait can provide an agronomic and selective advantage for this GM cotton plant when dicamba- and/or glufosinate ammonium-based herbicides are applied.

The applicant presented agronomic and phenotypic data on cotton MON 88701 (Section 3.2.1.2). The data set showed no changes in agronomic and phenotypic plant characteristics that would indicate altered fitness, persistence and invasiveness of cotton MON 88701.

In case of accidental release into the environment of viable seeds of cotton MON 88701 during transportation and processing, establishment and survival of this GM cotton in the European Union (EU) will be limited by the biotic and abiotic factors described above. Should MON 88701 plants be exposed to dicamba- and/or glufosinate ammonium-based herbicides, they would be likely to exhibit a selective advantage that could increase their local occurrence. As the occurrence is expected to be transient, this will not result in different environmental impacts compared to conventional cotton.

In addition to the data presented by the applicant, the GMO Panel is not aware of any scientific report of increased spread and establishment of GM cotton MON 88701 in regions where it is cultivated, and of any change in survival capacity, including overwintering.

The GMO Panel concludes that it is very unlikely that cotton MON 88701 will differ from conventional cotton varieties in its ability to survive or establish feral populations under European environmental conditions.

Effects of gene transfer

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

1) Plant-to-bacteria gene transfer[28]

Genomic plant DNA is a component of many food and feed products derived from cotton. It is well documented that DNA present in food and feed becomes substantially degraded during processing and

[27] Dossier: Part II – Section E3.1.
[28] Dossier: Part II – Sections E3.1 and E3.2.

digestion in the human or animal gastrointestinal tract. However, a low level of exposure of fragments of ingested DNA, including the recombinant fraction of such DNA, to bacteria in the digestive tract of humans, domesticated animals and other environments exposed to the GM plant or plant material is expected.

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

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

Cotton MON 88701 contains four recombinant DNA sequences originating from bacteria, i.e. (1) the codon-optimised coding sequence for the DMO protein from *S. maltophilia*; (2) the coding sequence for the *pat* gene of *S. hygroscopicus*; (3) the 3' UTR sequence of the *nos* gene from *A. tumefaciens*; and (4) a sequence of approximately 260 bp of the DNA region from *A. tumefaciens* containing the left border of the PV-GHHT6997 vector sequence used for transfer of the T-DNA.

Bioinformatic analysis of the inserted DNA and flanking regions did not identify sufficient sequence identity with bacterial DNA for the codon-optimised gene encoding for DMO, but revealed sequence identities of the *pat* gene from *S. hygroscopicus*. Sequence identities of the *nos* gene and the left border region with respective sequences of Ti plasmids of *A. tumefaciens* were also found, confirming their origin. However, for the latter, both DNA sequences do not originate from the same Ti plasmids, since the left boarder sequence has identity with DNA from the octopine Ti plasmid pT15955 while the nos sequence has identity with a sequence of nopaline Ti plasmid. Thus, the identified sequence identities do not indicate any potential for double homologous recombination, which would potentially result in a transfer of DNA located between then. Due to the sequence identity, however, substitutive recombination, replacing natural variants of DNA sequences in respective bacterial hosts, could theoretically be facilitated.

In addition to homology-based recombination processes, non-homologous (illegitimate) recombination that does not require the presence of DNA similarity between the recombining DNA molecules is theoretically possible. However, illegitimate recombination events have not been detected in studies that have exposed bacteria to high concentrations of GM plant DNA (EFSA, 2009). Thus, this process, in comparison with homologous recombination, is not considered to contribute significantly to horizontal gene transfer events.

In case of products of cotton MON 88701 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). Because of the restricted dietary amounts of cotton MON 88701, effects of feed processing and degradation in the gastrointestinal tract and faeces, the manure of animals fed with cotton MON 88701 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 88701 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 harbouring genes with sufficient DNA sequence identity with DNA from cotton MON 88701. Both bacterial species, identified in this respect, i.e. *S. hygroscopicus* and *A. tumefaciens,* are not common bacterial inhabitants of the gastrointestinal tract, but they may occur in soil. As indicated above, the exposure to DNA in this environmental compartment is expected to be very low and only substitute recombination would be facilitated. Since substitutive recombination would only replace natural variants of the same genes, this process would not confer any new trait or selective advantage to the bacterial recipients.

In conclusion, the GMO Panel did not identify properties of the DNA inserted into cotton MON 88701 that would change its likelihood of horizontal transfer compared with other plant genes. Therefore, the GMO Panel concludes that the recombinant DNA in cotton MON 88701 does not represent an environmental risk in relation to its potential for horizontal transfer to bacteria.

2) Plant-to-plant gene transfer

Considering the scope of the application EFSA-GMO-NL-2013-114 and the biology of cotton, the potential of occasional feral GM cotton plants originating from seed import spills to transfer recombinant DNA to sexually cross-compatible plants is assessed.

Cotton is predominantly an annual self-pollinating crop, although cross-pollination can occur at low frequencies in the presence of insect pollinators (such as wild bees, honeybees, bumblebees) (OECD, 2008).

The extent of cross-pollination will mainly depend on the scale of accidental release during transportation and processing, and the successful establishment and subsequent flowering of GM cotton plants. For cotton, no wild relatives have been reported in Europe; therefore, any vertical gene transfer is limited to cultivated (*G. hirsutum* and *G. herbaceum*) and feral cotton plants. However, gene transfer to *G. herbaceum* is considered unlikely due to the difference in ploidy level.

The occurrence of feral GM cotton is expected to be limited. For plant-to-plant gene transfer to occur, imported cottonseeds need to be processed outside the importing ports, transported into regions of cotton production in Europe, spilled during transportation, germinate and develop into plants in the very close vicinity of cotton fields, and there needs to be an overlap of flowering periods and environmental conditions favouring cross-pollination. It must be noted that most cottonseeds are processed in the countries of production or in ports of importation.

In conclusion, the GMO Panel considers that the likelihood of environmental effects as a consequence of the spread of genes from cotton MON 88701 in Europe will not differ from that of conventional cotton, even after exposure to dicamba- and glufosinate ammonium-based herbicides.

Interactions of the GM plant with target organisms[29]

Considering the scope of application EFSA-GMO-NL-2013-114, and the absence of target organisms, potential interactions of feral cotton MON 88701 plants arising from seed import spills with target organisms are not considered a relevant issue by the GMO Panel.

Interactions of the GM plant with non-target organisms[30]

Considering the scope of application EFSA-GMO-NL-2013-114, and the low level of exposure to the environment, potential interactions of spilled seeds or occasional feral cotton MON 88701 plants arising from seed import spills with non-target organisms are not considered a relevant issue by the GMO Panel.

Interactions with the abiotic environment and biochemical cycles[31]

Considering the scope of application EFSA-GMO-NL-2013-114, and the low level of exposure to the environment, potential interactions of occasional feral cotton MON 88701 plants arising from seed import spills with the abiotic environment and biogeochemical cycles are not considered a relevant issue by the GMO Panel.

3.4.1.2. Post-market environmental monitoring[32]

The objectives of a PMEM plan, according to Annex VII of Directive 2001/18/EC are: (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the ERA are correct; and (2) to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment that 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 GMO Panel gives its opinion on the scientific content of the PMEM plan provided by the applicant (EFSA GMO Panel, 2011b).

The PMEM plan proposed by the applicant for cotton MON 88701 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 information recorded by the various operators; and (3) the review of relevant scientific publications retrieved from literature searches (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 period.

[29] Dossier: Part II – Section E3.3.
[30] Dossier: Part II – Section E3.4.
[31] Dossier: Part II – Section E3.6.
[32] Dossier: Part II – Section E4.

The GMO Panel considers the scope of the PMEM plan provided by the applicant is consistent with the scope of cotton MON 88701. As the scope does not cover cultivation and potential adverse environmental effects from cotton MON 88701 were not identified, no case-specific monitoring is necessary. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

3.4.2. Conclusion on the environmental risk assessment and monitoring plan

In the case of accidental release into the environment of viable seeds of cotton MON 88701, there are no indications of an increased likelihood of establishment and spread of occasional feral cotton MON 88701 plants, unless these plants were exposed to the intended herbicides. The GMO Panel is of the opinion that this will not result in different environmental impacts compared to conventional cotton. Considering the scope of the application EFSA-GMO-NL-2013-114, interactions of cotton MON 88701 with the biotic and abiotic environment are not considered to be relevant issues. The GMO Panel concludes that the recombinant DNA in cotton MON 88701 does not represent an environmental risk in relation to its potential for horizontal transfer to bacteria. Therefore, considering the introduced traits, the outcome of the agronomic and phenotypic characterisation, the routes of exposure and the limited exposure levels, the GMO Panel concludes that cotton MON 88701 would not raise safety concerns in the event of accidental release of viable GM cottonseeds into the environment.

The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of cotton MON 88701 and the GMO Panel guidelines on the PMEM of GM plants (EFSA GMO Panel, 2011b).

4. Conclusions

The GMO Panel was asked to carry out a scientific assessment of cotton MON 88701 for import, processing, and food and feed uses in accordance with Regulation (EC) No 1829/2003.

The molecular characterisation data and bioinformatics analyses did not identify issues requiring further assessment for food/feed safety.

The GMO Panel concludes that none of the differences identified in the agronomic and phenotypic characteristics of cotton MON 88701 required further assessment regarding environmental and food and feed safety. Compositional results fulfilling the minimum requirements regarding the number of field trials locations and replications were reported for only three out of the eight sites. The GMO Panel is therefore not in the position to complete the assessment of the compositional analysis. Moreover, as no 28-day toxicity study in rodents on the MON 88701 DMO protein was provided, the GMO Panel is not in the position to complete the safety assessment of this protein in cotton MON 88701. Consequently, the GMO Panel cannot complete the toxicological, allergenicity and nutritional assessment of food/feed derived from cotton MON 88701. The safety assessment identified no concerns regarding the potential toxicity and allergenicity of the PAT protein newly expressed in cotton MON 88701.

The GMO Panel concludes that there is a very low likelihood of environmental effects resulting from the accidental release of viable seeds from cotton MON 88701 into the environment. Considering the scope of the application with regard to food and feed uses, interactions with the biotic and abiotic environment are not considered an issue. Risks associated with an unlikely, but theoretically possible, horizontal gene transfer from cotton MON 88701 to bacteria have not been identified. The scope of the PMEM plan provided by the applicant is in line with the intended uses of cotton MON 88701.

In conclusion, in the absence of an appropriate comparative assessment and an appropriate assessment of the MON 88701 DMO protein, the GMO Panel is not in a position to complete its food/feed risk assessment of cotton MON 88701. However, the GMO Panel concludes that the cotton MON 88701 is unlikely to have any adverse effect on the environment in the context of the scope of the application.

Documentation provided to EFSA

1) Letter from the Competent Authority of the Netherlands received on 13 February 2013 concerning a request for placing on the market of genetically modified cotton MON 88701 submitted by Monsanto Europe S.A./N.V. in accordance with Regulation (EC) No 1829/2003 (application reference EFSA-GMO-NL-2013-114).
2) Acknowledgement letter dated 05 March 2013 from EFSA to the Competent Authority of the Netherlands.

3) Letter from EFSA to applicant dated 27 March 2013 requesting additional information under completeness check.
4) Letter from applicant to EFSA received on 24 April 2013 additional information under completeness check.
5) Letter from EFSA to applicant dated 21 May 2013 requesting additional information under completeness check.
6) Email from applicant to EFSA, received on 4 June 2013 providing clarifications.
7) Letter from EFSA to applicant dated 25 June 2013 delivering the 'Statement of Validity' of application EFSA-GMO-NL-2013-114 for placing on the market of genetically modified cotton MON 88701 submitted by Monsanto Europe S.A./N.V. in accordance with Regulation (EC) No 1829/2003.
8) Letter from EFSA to applicant dated 13 September 2013 requesting additional information and stopping the clock.
9) Letter from EFSA to applicant dated 12 November 2013 requesting additional information and maintaining the clock stopped.
10) Letter from EFSA to applicant dated 19 December 2013 requesting additional information and maintaining the clock stopped.
11) Letter from applicant to EFSA received on 24 January 2014 providing additional information.
12) Letter from applicant to EFSA received on 10 February 2014 providing additional information.
13) Letter from EFSA to applicant dated 19 May 2014 requesting additional information and maintaining the clock stopped.
14) Letter from EFSA to applicant dated 5 June 2014 requesting additional information and maintaining the clock stopped.
15) Letter from applicant to EFSA received on 1 August 2014 providing additional information.
16) Letter from applicant to EFSA received on 22 September 2014 providing additional information.
17) Letter from EFSA to applicant dated 18 December 2014 requesting additional information and maintaining the clock stopped.
18) Letter from applicant to EFSA received on 20 February 2015 providing additional information.
19) Letter from EFSA to applicant dated 3 June 2015 requesting additional information and maintaining the clock stopped.
20) Letter from applicant to EFSA received on 13 August 2015 providing additional information.
21) Letter from EFSA to applicant dated 9 September 2015 requesting additional information and maintaining the clock stopped.
22) Letter from applicant to EFSA, received on 7 October 2015 requesting clarifications.
23) Letter from EFSA to applicant, dated 26 October 2015 providing clarifications.
24) Letter from applicant to EFSA received on 11 January 2016 providing additional information and requesting further clarifications.
25) Letter from EFSA to applicant, dated 12 February 2016 providing clarifications.
26) Letter from EFSA to applicant dated 23 March 2016 requesting additional information and maintaining the clock stopped.
27) Letter from applicant to EFSA received on 24 November 2016 providing additional information.
28) Email from EFSA to applicant dated 25 November 2016 re-starting the clock on 24 November 2016.
29) Letter from EFSA to applicant dated 12 December 2016 requesting additional information and stopping the clock.
30) Letter from applicant to EFSA received on 20 January 2017 providing additional information.
31) Email from EFSA to applicant dated 23 January 2017 re-starting the clock on 20 January 2017.

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Abbreviations

bp	base pair

Bw	body weight
CTP	chloroplast transit peptide
DAP	days after planting
DMO	dicamba mono-oxygenase
ELISA	enzyme-linked immunosorbent assay
ERA	environmental risk assessment
GM	genetically modified
GMO	genetically modified organism
GMO	Panel EFSA Panel on Genetically Modified Organisms
HGT	horizontal gene transfer
HR	homologous recombination
Hsp70	heat shock protein 70
IgE	immunoglobulin E
Nos	nopaline synthase
OECD	Organisation for Economic Co-operation and Development)
ORF	open reading frame
PAT	phosphinothricin *N*-acetyltransferase
PCR	polymerase chain reaction
PMEM	post-market environmental monitoring
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SIF	simulated intestinal fluid
TEV	Tobacco etch virus
UTR	untranslated region