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Scientific Opinion on an application by Dow Agrosciences LLC (EFSA-GMO-NL-2009-68) for placing on the market of cotton 281-24-236 × 3006-210-23 × MON 88913 for food and feed uses, import and processing under Regulation (EC) No 1829/2003

EFSA Panel on Genetically Modified Organisms (GMO)

Abstract

The Panel on Genetically Modified Organisms of the European Food Safety Authority (GMO Panel) previously assessed the three single events combined to produce a three-event stack cotton 281-24-236 × 3006-210-23 × MON 88913 and did not identify safety concerns. In this opinion, the GMO Panel assesses only the three-event stack cotton. No new data on the single events, leading to modification of the original conclusions on their safety, were identified. The combination of cotton events 281-24-236, 3006-210-23 and MON 88913 in the three-event stack cotton did not give rise to issues – based on the molecular, agronomic, phenotypic or compositional characteristics – regarding food and feed safety and nutrition. The combination of the newly expressed proteins in the three-event stack cotton did not raise concerns for human and animal health. Considering the introduced traits and the outcome of the comparative analysis, the routes of exposure and limited exposure levels, the GMO Panel concludes that this three-event stack cotton would not raise safety concerns in case of accidental release of viable cottonseeds into the environment. The post-market environmental monitoring plans provided by the applicant are in line with the scope of the three-event stack cotton. No post-market monitoring of food/feed derived from the three-event stack cotton is considered necessary. The GMO Panel concludes that the three-event stack cotton is as safe and as nutritious as its conventional counterpart in the context of its scope.

Keywords: GMO, cotton 281-24-236 × 3006-210-23 × MON 88913, Regulation (EC) 1829/2003, Cry1Ac, Cry1F, CP4 EPSPS, import and processing

Requestor: Competent Authority of the Netherlands

Question number: EFSA-Q-2009-00491

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Summary

Following the submission of application EFSA-GMO-NL-2009-68 under Regulation (EC) No 1829/2003[1] from Dow Agrosciences LLC, 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 insect-resistant and herbicide-tolerant genetically modified (GM) cotton 281-24-236 × 3006-210-23 × MON 88913 (hereafter referred to as 'three-event stack cotton'). The scope of application EFSA-GMO-NL-2009-68 is for food and feed uses, import and processing, but excludes cultivation within the European Union (EU).

The applicant restricted the scope of application EFSA-GMO-NL-2009-68 to the three-event stack cotton only. As cotton (*Gossypium hirsutum*) is predominantly a self-pollinator and the three-event stack cotton 281-24-236 × 3006-210-23 × MON 88913 is homozygous for all traits,[2] the produced and imported cottonseed of this GM cotton will contain all traits, and segregants are expected only at very low frequency.

In accordance with the GMO Panel guidance document applicable to this application (EFSA, 2007), *where all single events have been assessed, the risk assessment of stacked events should focus mainly on issues related to (a) stability, (b) expression of the events and (c) potential interactions between the events*. For application EFSA-GMO-NL-2009-68, previous assessments of the three single events (281-24-236, 3006-210-23 and MON 88913) provided a basis to evaluate the three-event stack cotton.

The three-event stack cotton 281-24-236 × 3006-210-23 × MON 88913 was produced by conventional crossing of the two-event stack cotton 281-24-236 × 3006-210-23, containing the previously assessed single cotton events 281-24-236, 3006-210-23 and MON 88913. The single events 281-24-236 (expressing Cry1F and phosphinothricin acetyltransferase (PAT) proteins) and 3006-210-23 (expressing Cry1Ac and PAT proteins), in the frame of the two-event stack cotton 281-24-236 × 3006-210-23, and MON 88913 (expressing two copies of the CP4 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein) were assessed previously and no concerns were identified. No safety issue was identified by updated bioinformatic analyses, nor reported by the applicant concerning the three single cotton events, since the publication of the respective scientific opinions. Consequently, the GMO Panel considers that its previous conclusions on the safety of the single cotton events remain valid (Section 3.2).

For the three-event stack cotton, the risk assessment included the molecular characterisation of the inserted DNA and the analysis of the protein expression. 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 characteristics. Evaluation of environmental impacts and of the post-market environmental monitoring (PMEM) plan was also undertaken.

The molecular data establish that the events stacked in cotton 281-24-236 × 3006-210-23 × MON 88913 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins are similar in the three-event stack cotton, and the two GM parental lines, i.e. the two-event stack (281-24-236 × 3006-210-23) and single event (MON 88913). There is no indication of an interaction that may affect the integrity of the events and the levels of the newly expressed proteins in this stack.

The combination of the newly expressed proteins in the three-event stack cotton did not raise concerns for human and animal health. The combination of cotton events 281-24-236, 3006-210-23 and MON 88913 in the three-event stack cotton did not give rise to issues – based on the agronomic, phenotypic or compositional characteristics – regarding food and feed safety and nutrition.

Considering the introduced traits, the outcome of the comparative analysis, the routes of exposure and the limited exposure levels, the GMO Panel concluded that this three-event stack cotton would not raise safety concerns in the event of accidental release of viable GM cottonseeds into the environment.

The GMO Panel is of the opinion that the PMEM plans provided by the applicant are in line with the scope of the three-event stack cotton and that post-market monitoring of food/feed derived from the three-event stack cotton is not considered necessary.

In conclusion, the GMO Panel concludes that the three-event stack cotton is as safe and as nutritious as its conventional counterpart in the context of its scope.

[1] 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.

[2] Dossier: Part I – Section A4.

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1. Introduction

1.1. Background

On 19 March 2009, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands application EFSA-GMO-NL-2009-68, for authorisation of genetically modified (GM) cotton 281-24-236 × 3006-210-23 × MON 88913 for food and feed uses, import and processing submitted by Dow AgroSciences LLC within the framework of Regulation (EC) No 1829/2003 on genetically modified food and feed.

The scope defined by the applicant at the time of submission was all food and feed products containing, consisting or produced from cotton 281-24-236 × 3006-210-23 × MON 88913. Cotton (*Gossypium hirsutum*) is predominantly a self-pollinator and the stacked cotton 281-24-236 × 3006-210-23 × MON 88913 is homozygous for all traits.[3] Therefore, the produced and imported cottonseed of this GM cotton will contain all traits, and segregants are expected only at very low frequency. The scope of this application therefore only covers the cotton 281-24-236 × 3006-210-23 × MON 88913. The EFSA Panel on Genetically Modified Organisms (EFSA GMO Panel) assessed application EFSA-GMO-NL-2009-68 in the light of the scope as defined by the applicant.

After receiving the application EFSA-GMO-NL-2009-68 and in accordance with Articles 5(2)(b) and 17(2)(b) of Regulation (EC) No 1829/2003, EFSA informed Member States and the European Commission (EC), and made the summary of the application available to the public on the EFSA website. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of Regulation (EC) No 1829/2003. On 17 May 2010, 2 December 2010 and 9 February 2011, EFSA received additional information (requested on 28 April 2009, 4 June 2010 and 17 January 2011, respectively). On 3 March 2011, EFSA declared the application 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 EC, 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 opening of the Member State commenting period (until 5 December 2013) to make their opinion known.

The GMO Panel carried out an evaluation of the scientific risk assessment of GM cotton 281-24-236 × 3006-210-23 × MON 88913. On 28 September 2012, the EURL-GMFF requested additional information to the applicant. On 23 September 2013, the applicant provided the requested information to the EURL-GMFF. On 17 January 2014, 31 October 2014, 25 March 2015, 14 October 2015 and 25 November 2015, the GMO Panel requested additional information from the applicants. The applicants provided the requested information on 22 September 2014, 16 December 2014, 11 December 2015, 26 January 2016 and on 16 February 2016. The applicant also spontaneously submitted additional information on 24 February 2015, 2 March 2015 and on 12 June 2015. The clock of the application was stopped from 4 March 2011 to 5 August 2013 due to the pending assessment of the single-event cotton MON 88913 (application reference EFSA-GMO-UK-2007-41).

In giving its scientific opinion 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 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 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 risk assessment of cotton 281-24-236 × 3006-210-23 × MON 88913 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003.

[3] Dossier: Part I – Section A4.

[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.

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/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-2009-68, 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 a scientific risk assessment of cotton 281-24-236 × 3006-210-23 × MON 88913 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, 2006a, 2007; EFSA GMO Panel, 2011a), for the environmental risk assessment (ERA) of GM plants (EFSA GMO Panel, 2010b) and for 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 EFSA's overall opinion[5] and were taken into consideration during the scientific risk assessment.

3. Assessment

3.1. Introduction

Application EFSA-GMO-NL-2009-68 covers a three-event stack cotton 281-24-236 × 3006-210-23 × MON 88913 produced by conventional crossing. The scope of this application is for food and feed uses, import and processing, but excludes cultivation within the European Union (EU).

Cotton 281-24-236 × 3006-210-23 × MON 88913 was developed to confer resistance against certain lepidopteran target pests and tolerance to glyphosate-based herbicides. Resistance to lepidopteran target pests is achieved by the expression of Cry1Ac and Cry1F. Tolerance to glyphosate is achieved by expression of the CP4 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS). In addition, this cotton also expresses the phosphinothricin acetyltransferase (PAT) protein which confers tolerance to glufosinate ammonium-based herbicides. However, the applicant indicates that the tolerance achieved is not sufficient to be used under field conditions.

The three-event stack cotton 281-24-236 × 3006-210-23 × MON 88913 was produced by conventional crossing of the two-event stack cotton 281-24-236 × 3006-210-23, containing the previously assessed single cotton events 281-24-236, 3006-210-23 and MON 88913 (see Table 1), for which no concerns for human and animal health or environmental safety were identified.

The EFSA guidance applicable to this application establishes that *where all single events have been assessed, the risk assessment of stacked events should focus mainly on issues related to (a) stability, (b) expression of the events and (c) potential interactions between the events* (EFSA, 2006a, 2007). Additional information received after May 2011 was assessed in accordance with the 2011 guidance (EFSA GMO Panel, 2011a).

[5] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2016-00198

Table 1: Single cotton events and cotton stacks already assessed by the GMO Panel

Event	Application	EFSA Scientific Opinion
MON 88913	EFSA-GMO-UK-2007-41	2013
281-24-236 × 3006-210-23 (containing the single events 281-24-236 and 3006-210-23)	EFSA-GMO-NL-2005-16	2010a

GMO Panel: Panel on Genetically Modified Organisms of the European Food Safety Authority.

3.2. Updated information on single events

Since the publication of the scientific opinions on the single and parental lines of the three-event stack cotton by the EFSA GMO Panel (2010a, 2013), no safety issue pertaining to the three single events has been reported by the applicant.

Updated bioinformatic analyses on the junction regions for events 281-24-236, 3006-210-23 and MON 88913 confirmed that no known endogenous genes were disrupted by any of the inserts for events 3006-210-23 and MON 88913. Updated bioinformatic analyses for event 281-24-236 confirmed that its insertion occurred in the 3′ untranslated region (UTR) of a gibberellin 20-oxidase gene. The potential interruption of this gene was assessed in the framework of application EFSA-GMO-NL-2005-16 (EFSA GMO Panel, 2010a). Since the publication of EFSA's opinion on application EFSA-GMO-NL-2005-16, new information became available suggesting that this gibberellin 20-oxidase gene is associated with fibre development (Xiao et al., 2010). The insertion of event 281-24-236 in this gibberellin 20-oxidase gene might have affected its expression. The GMO Panel considered this new information from the literature in its comparative assessment (Section 3.3.2).

Updated bioinformatic analyses of the amino acid sequence of the newly expressed Cry1Ac, Cry1F, CP4 EPSPS and PAT proteins revealed no significant similarities to toxins and allergens.[6] In addition, updated bioinformatics analyses of the newly created open reading frames (ORFs) within the inserts and at their junctions did not indicate significant similarities to toxins and allergens either.[7]

Based on the above information, the GMO Panel considers that its previous conclusions on the safety of the single cotton events remain valid.

3.3. Risk assessment of the three-event stack cotton 281-24-236 × 3006-210-23 × MON 88913

3.3.1. Molecular characterisation

Possible interactions that would affect the integrity of the events, protein expression level or the biological function conferred by the individual inserts are considered.

3.3.1.1. Genetic elements and biological functions of the inserts[8]

The two-event stack cotton 281-24-236 × 3006-210-23 and MON 88913 are combined by conventional crossing to produce the three-event stack cotton 281-24-236 × 3006-210-23 × MON 88913. The structures of the inserts introduced into the three-event stack cotton are described in detail in the respective EFSA scientific opinions and no new genetic modifications were involved. Genetic elements in the expression cassettes of the single events are summarised in Table 2.

Intended effects of the inserts in cotton 281-24-236 × 3006-210-23 × MON 88913 are summarised in Table 3.

Based on the known biological function (Table 3) of the newly expressed proteins, the only foreseen interactions at the biological level are between the two Cry proteins in susceptible insects.

[6] Additional information: 24/2/2015, 12/6/2015.
[7] Additional information: 24/2/2015, 12/6/2015.
[8] Dossier: Part I – Section D.

Table 2: Genetic elements in the expression cassettes of the events stacked in cotton 281-24-236 × 3006-210-23 × MON 88913

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
281-24-236	*Ubi1* (exon1-intron1) (*Zea mays*)[a]	–	No	*pat* (*Streptomyces viridochromogenes*)	orf25 polyA (*Agrobacterium tumefaciens*)
	4ocsΔmas2′ (pTi15955)	–	No	*cry1F* (*Bacillus thuringiensis* var. *aizawai*)	orf25 polyA (*A. tumefaciens*)
3006-210-23	*4ocsAtuMas* (pTi15955)	–	No	*pat* (*S. viridochromogenes*)	orf25 polyA (*A. tumefaciens*)
	Ubi1 (exon1-intron1) (*Z. mays*)	–	No	*cry1Ac* (*B. thuringiensis* var. *kurstaki*	orf25 polyA (*A. tumefaciens*)
MON 88913	*FMV* (*figwort mosaic virus*)/*Tsf1* (*Arabidopsis thaliana*)	*Tsf1* (*A. thaliana*)	CTP2 (*A. thaliana*)	cp4 *epsps* (*Agrobacterium* sp. strain CP4)	*E9* (*Pisum sativum*)
	35S (*Cauliflower mosaic virus*)/act8 (*A. thaliana*)	*act8* *(A. thaliana)*	CTP2 *(A. thaliana)*	cp4 *epsps* *(Agrobacterium* sp. strain CP4*)*	*E9* (*P. sativum*)

(a): Source of genetic information.

Table 3: Characteristics and intended effects of the events stacked in cotton 281-24-236 × 3006-210-23 × MON 88913

Event	Protein	Donor organism and biological function	Intended effects in GM plant
281-24-236	PAT	Based on a gene from *Streptomyces viridochromogenes*. Phosphinothricin-acetyl-transferase (PAT) enzyme acetylates L-glufosinate-ammonium and thereby confers tolerance to phosphinothricin-based herbicides (Eckes et al., 1989)	Expression of PAT in cotton 281 24-236 confers tolerance to glufosinate ammonium-based herbicides
	Cry1F	Based on genes from *Bacillus thuringiensis* subsp. *aizawai*. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (cry) genes[a]	Cotton 281-24-236 expresses a chimeric, codon-optimised, full length *cry1F* gene which was modified to enhance its expression in plants. Cry1F is a protein toxic to certain lepidopteran larvae feeding on cotton
3006-210-23	PAT	Based on a gene from *S. viridochromogenes* Phosphinothricin-acetyl-transferase (PAT) enzyme confers resistance to the antibiotic bialaphos (Eckes et al., 1989)	Expression of PAT in cotton 3006-210-23 confers tolerance to glufosinate ammonium-based herbicides
	Cry1Ac	Based on genes from *B. thuringiensis* var. *kurstaki*. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes[a]	Cotton 3006-210-23 expresses a chimeric, codon-optimised, full-length *cry1Ac* gene which was modified to enhance its expression in plants. Cry1Ac is a chimeric protein toxic to certain lepidopteran larvae feeding on cotton
MON 88913	CP4 EPSPS	Based on a gene from *Agrobacterium* strain CP4. 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) is an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Herrmann, 1995)	The bacterial CP4 EPSPS confers tolerance to glyphosate-based herbicides as it has lower affinity towards glyphosate than the plant endogenous enzyme

GM: genetically modified.
(a): Dossier: Part I – Section C3 and D1.

3.3.1.2. Integrity of the events in the three-event stack

The genetic stability of the inserted DNA over multiple generations in the MON 88913 cotton event was demonstrated previously (EFSA GMO Panel, 2013). Integrity of the events in the two-event stack cotton 281-24-236 × 3006-210-23 stack was demonstrated previously (EFSA GMO Panel, 2010a). Integrity of these events was demonstrated in the three-event stack cotton[9] by Southern analyses.

3.3.1.3. Information on the expression of the inserts[10]

Plants were grown at five locations (three replicate plots) under field conditions in the USA in 2005. The levels of the Cry1F, Cry1Ac, PAT and CP4 EPSPS proteins in the three-event stack cotton, and the GM parental lines, i.e. the two-event stack 281-24-236 × 3006-210-23 and the single event MON 88913 were analysed by enzyme-linked immunosorbent assay (ELISA). Protein levels were determined in leaf, pollen, whole plant, root, cottonseed and processed fractions. Data on cottonseeds are reported and discussed below (Table 4). Cry1F, Cry1Ac, PAT and CP4 EPSPS protein levels in the three-event stack cotton were similar to the corresponding levels in the two-event stack and single-event cotton plants.

Table 4: Means, standard deviations and ranges (n = 15) of protein levels in cottonseeds (μg/g dry weight) from cotton 281-24-236 × 3006-210-23 × MON 88913, 281-24-236 × 3006-210-23 and MON 88913

Event/ protein	281-24-236 × 3006-210-23 × MON 88913 unsprayed	281-24-236 × 3006-210-23 × MON 88913 sprayed with glyphosate	281-24-236 × 3006-210-23 unsprayed	MON 88913 unsprayed
Cry1F	3.15[(a)] ± 0.48[(b)]	2.70 ± 0.41	2.75 ± 0.45	–
	2.38–4.15[(c)]	2.20–3.39	1.96–3.40	
Cry1Ac	0.90 ± 0.21	0.89 ± 0.20	0.98 ± 0.27	–
	0.54–1.30	0.60–1.31	0.60–1.62	
PAT	0.50 ± 0.14	0.50 ± 0.14	0.37 ± 0.13	–
	0.33–0.76	0.28–0.74	0.15–0.59	
CP4 EPSPS	178 ± 23.0	175 ± 31.2	–	171 ± 25.6
	146–233	130–246		132–226

–: not assayed.
(a): Mean.
(b): Standard deviation.
(c): Range.

3.3.1.4. Conclusion

The molecular data establish that the events stacked in cotton 281-24-236 × 3006-210-23 × MON 88913 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins are similar in the three-event stack and the GM parental lines. Therefore, there is no indication of an interaction that may affect the integrity of the events and the levels of the newly expressed proteins in this stack.

Based on the known biological function (Table 3) of the newly expressed proteins, the only foreseen interactions at the biological level are between the two Cry proteins in susceptible insects, which will be dealt with in Section 3.3.4.

3.3.2. Comparative analyses

3.3.2.1. Choice of comparator and production of material for the comparative assessment

The application EFSA-GMO-NL-2009-68 presented agronomic and phenotypic characteristics data on cotton and compositional data on cottonseeds collected in field trials performed in the USA in 2005.

[9] Dossier: Part I – Section D5.
[10] Dossier: Part I – Section D3.

The field trials included the stacked cotton 281-24-236 × 3006-210-23 × MON 88913 and its non-GM comparator PSC355. The non-GM comparator PSC355 had a similar genetic background as the three-event stack cotton 281-24-236 × 3006-210-23 × MON 88913 and was considered a suitable conventional counterpart by the GMO Panel.

The field trials were conducted during the 2005 growing season in five locations in the USA, representing typical cotton-growing regions.[11] A randomised block design with three replications of cotton 281-24-236 × 3006-210-23 × MON 88913 and its conventional counterpart was used. The conventional counterpart PSC355 received location-specific maintenance pesticide management only; while cotton 281-24-236 × 3006-210-23 × MON 88913 received maintenance management with and without additional application of glyphosate-based herbicides. The single event MON 88913 and the two-event stack cotton 281-24-236 × 3006-210-23 were included in the field trials as additional comparators.

3.3.2.2. Agronomic and phenotypic analysis

Measurements of agronomic and phenotypic characteristics included endpoints related to plant growth and morphology at different life stages, reproduction, agricultural productivity and disease susceptibility.[12]

In order to test for differences between the three-event stack cotton and its conventional counterpart, an analysis of variance was applied across the field trial sites.[13]

Statistically significant differences were observed between the three-event stack cotton 281-24-236 × 3006-210-23 × MON 88913 (with maintenance management treatment only) and its conventional counterpart for the following endpoints: total nodes (14 days), node of first white flower (42 days), elongation and leaf (Table 5).[14]

Table 5: Agronomic and phenotypic endpoints for which a statistically significant difference was observed between the cotton 281-24-236 × 3006-210-23 × MON 88913 (treated or not treated with the intended herbicide) and its conventional counterpart PSC355

Endpoint	Conventional counterpart PSC355	Cotton 281-24-236 × 3006-210-23 × MON 88913	
		Not treated with the intended herbicide	Treated with the intended herbicide
Total nodes – 14 days	15.0	15.7*	15.4
Node of first white flower – 42 days	13.2	14.0*	13.9
Micronaire (mic units)	4.77	4.53	4.44*
Fibre elongation (%)	6.77	6.94*	7.12*
Leaf (thrash)	4.40	3.80*	4.13

Significantly different entries are marked with an asterisk.

Statistically significant differences were observed between three-event stack cotton 281-24-236 × 3006-210-23 × MON 88913 (treated with maintenance and glyphosate-based herbicides) compared to its conventional counterpart for the following endpoints: fibre micronaire and fibre elongation (Table 5). The difference in fibre elongation may be related to the interruption of the gibberellin-20-oxidase gene (see Section 3.2).

The significant differences in Table 5 are further assessed for their potential environmental impact in Section 3.3.4.

3.3.2.3. A compositional analysis

Comparative compositional analysis was performed on delinted cottonseed samples obtained from the three-event stack cotton 281-24-236 × 3006-210-23 × MON 88913 and its conventional

[11] Dossier: Part I – Section 7.1 (Study 050006.03).

[12] Plant stand, plant vigour, crop injury (after first, second, third and fourth applications), node of first white flower (14, 28 and 42 days), plant height (14, 28 and 42 days), total nodes (14, 28 and 42 days), number of bolls, number of open bolls, open % open bolls, disease incidence, insect damage, weight of seed cotton and weight of ginned cotton, micronaire, length, uniformity, strength, elongation, colour (Rd and +b), trash (leaf)).

[13] A linear mixed model was used, where the overall mean and the genotype effect were fixed effects. The random effects (apart from residual error) were location, block-within-location and location-by-genotype.

[14] Leaf (thrash) is a measure of the amount of non-lint materials in cotton, such as leaf and bark from the cotton plant and used for fibre quality.

counterpart PSC355. Delinted cottonseed represents the starting material for food and feed products produced from cotton.

Cottonseed samples were analysed for a total of 79 compositional parameters, selected in line with the Organisation for Economic Co-operation and Development (OECD) recommendations (OECD, 2004). Seventeen constituents with values below the limit of quantification were excluded from the statistical analysis.[15] For the remaining 62 endpoints,[16] in order to test for differences between the stacked cotton and its conventional counterpart, an analysis of variance was applied across the field trial sites.[17]

Statistically significant differences in delinted cottonseed between cotton 281-24-236 × 3006-210-23 × MON 88913 (not treated with the intended herbicides) and the conventional counterpart were identified for carbohydrates by calculation, gross energy by calculation (endpoint 'calories'), protein, calcium, glutamic acid and valine (Table 6).[18] All levels in the three-event stack cotton 281-24-236 × 3006-210-23 × MON 88913 fell within the range of non-GM cottonseeds reported in the literature (Berberich et al., 1996, OECD, 2009) and do not raise a food and feed safety concern.

Table 6: Compositional endpoints in delinted cottonseeds (from USA 2005 field trials) for which a statistically significant difference was identified between the three-event stack cotton 281-24-236 × 3006-210-23 × MON 88913 (treated or not treated with the intended herbicide) and its conventional counterpart PSC355

Parameter	Conventional counterpart PSC355	Cotton 281-24-236 × 3006-210-23 × MON 88913	
		Not treated with the intended herbicide	Treated with the intended herbicide
Protein (% dw)	24.3	26.0*	25.8*
Carbohydrate (% dw)	50.2	47.1*	47.8
Gross energy (MJ/kg dw)(a)	20.35	20.65*	20.56
Calcium (mg/kg dw)	1370	1220*	1220*
Glutamic acid (% dw)	5.37	5.61*	5.54
Valine (% dw)	1.09	1.14*	1.12
Sterculic acid (% FA)	0.134	0.129	0.106*
Malvalic acid (% FA)	0.229	0.228	0.184*

dw: dry weight; % FA: percentage of total fatty acid content.
Significantly different entries are marked with an asterisk.
(a): The applicant expressed gross energy (endpoint 'calories') in kcal/kg dw. In the present Opinion, these values have been converted into MJ/kg dw.

Statistically significant differences in delinted cottonseed between cotton 281-24-236 × 3006-210-23 × MON 88913 (treated with the intended herbicides) and the conventional counterpart were observed for protein content, calcium, sterculic acid and malvalic acid (Table 6). Except for sterculic acid, all levels in the three-event stack cotton 281-24-236 × 3006-210-23 × MON 88913 fell within the range of non-GM cottonseeds reported in the literature (Berberich et al., 1996, OECD, 2009). Sterculic acid is an

[15] The constituents were: molybdenum, selenium, caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristoleic acid (C14:1), pentadecanoic acid (C15:0), pentadecenoic acid (C15:1), heptadecanoic acid (C17:0), γ-linolenic acid (C18:3), eicosenoic acid (C20:1), eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3), arachidonic acid (C20:4), β-carotene, β-tocopherol and δ-tocopherol.

[16] Proximates and fibre (ash, fat, moisture, total protein, carbohydrates by calculation, calories (gross energy) by calculation, crude fibre, acid detergent fibre (ADF), neutral detergent fibre (NDF)), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, sulphur, zinc), amino acids (aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine, tryptophan), fatty acids (myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (20:0) and behenic acid (C22:0)), vitamins (thiamin, riboflavin, panthotenic acid, pyridoxine, ascorbic acid, folate, niacin), tocopherols (α-, γ- and total), sterculic acid, malvalic acid, dihydrosterculic acid, gossypol (total and free).

[17] A linear mixed model was used, where the overall mean and the genotype effect were fixed effects. The random effects (apart from residual error) were location, block-within-location and location-by-genotype.

[18] Carbohydrates and gross energy are calculated values, derived from the results obtained for the other proximates (ash, protein and total fat): the increase in protein content and gross energy in the three-event stack cotton 281-24-236 × 3006-210-23 × MON8891 is correlated with the decrease in carbohydrate levels.

anti-nutrient; the level in cotton 281-24-236 × 3006-210-23 × MON 88913 was lower than in its conventional counterpart and therefore it was considered by the GMO Panel to be of no food and feed safety concern.

3.3.2.4. Conclusion

The GMO Panel concludes that none of the differences identified in the agronomic and phenotypic characteristics and composition of seeds from the three-event stack cotton 281-24-236 × 3006-210-23 × MON 88913 needs further assessment regarding food and feed safety.

The differences observed for some agronomic and phenotypic characteristics are further assessed for their potential environmental impact in Section 3.3.4.

3.3.3. Food and feed safety assessment

3.3.3.1. Effect of processing[19]

Based on the outcome of the comparative assessment, processing of the three-event stack cotton into food and feed products is not expected to result in products being different from those of commercial non-GM cotton varieties.

Compositional data were obtained on processed products (toasted meal and refined oil) of cottonseeds from four out of five locations of the field trials carried out in the USA in 2005. The samples of the three-event stack cotton 281-24-236 × 3006-210-23 × MON 88913 and its conventional counterpart PSC355 from an individual location were harvested, pooled and processed. Toasted meal was analysed for proximates, fibre, amino acids, minerals, free and total gossypol, cyclopropenoid fatty acids and phytic acid.[20] Refined oil was analysed for fatty acids, tocopherols, total gossypol and cyclopropenoid fatty acids.[21] The values obtained for the composition of products derived from the three-event stack cotton 281-24-236 × 3006-210-23 × MON 88913 were in the same ranges previously described (e.g. Forster and Calhoun, 1995; Berberich et al., 1996; OECD, 2009). The GMO Panel concludes that the differences observed in the analysis of processed products derived from the three-event stack cotton 281-24-236 × 3006-210-23 × MON 88913 and its conventional counterpart do not need further assessment regarding food and feed safety.

In the same processing study, the levels of the newly expressed proteins Cry1F, Cry1Ac, CP4 EPSPS and PAT were also measured. Except for the PAT protein, all other proteins were identified in toasted meal and hulls but none of them were found in refined oil derived from the three-event stack cotton 281-24-236 × 3006-210-23 × MON 88913.

3.3.3.2. Toxicology

Toxicological assessment of newly expressed proteins

Four proteins are newly expressed in the three-event stack cotton 281-24-236 × 3006-210-23 × MON 88913 (Section 3.3.1). The GMO Panel has previously assessed these proteins individually in the context of the single event cotton MON 88913 and the two-event stack cotton 281-24-236 × 3006-210-23, and no safety concerns were identified.

The two enzymatic proteins (PAT, CP4EPSPS) act on unrelated substrates and are not expected to interact. The two insecticidal proteins (Cry1F, Cry1Ac) act through cellular receptors found in target insect species. It is reported that the gastrointestinal tract of mammals, including humans, lacks receptors with specific high affinity to Cry proteins (Hammond et al., 2013; Koch et al., 2015).

On the basis of the known biological function of the individual newly expressed proteins (Table 3), there is currently no expectation for possible interactions relevant to the food and feed safety assessment of the three-event stack cotton 281-24-236 × 3006-210-23 × MON 88913.

The GMO Panel concludes that there are no safety concerns to human and animal health related to the newly expressed proteins Cry1F, Cry1Ac, PAT and CP4EPSPS in the three-event stack cotton 281-24-236 × 3006-210-23 × MON 88913.

[19] Dossier: Part I – Section D7.6.

[20] Toasted meal: significantly lower levels in iron content (non-treated and treated with the intended herbicide) derived from the three-event stack and higher levels of the amino acids proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine and arginine in the material derived from the non-treated three-stacked event.

[21] Refined oil: significantly lower levels of palmitoleic acid, stearic acid, oleic acid, arachidic acid and behenic acid only observed in refined oil derived from the treated three-event stack cotton 281-24-236 × 3006-210-23 × MON 88913.

Toxicological assessment of components other than newly expressed proteins

The three-event stack cotton did not show any compositional difference from its conventional counterpart that would require further toxicological assessment (see Section 3.3.2).

However, the GMO Panel noted that the free gossypol content in raw cottonseeds of the three-event stack cotton 281-24-236 × 3006-210-23 × MON 88913 and its conventional counterpart was higher than the limits set in Directive 2002/32 EC[22] (5,000 mg/kg as fed) on undesirable substances in feed materials.

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

No animal studies with cotton 281-24-236 × 3006-210-23 × MON 88913 were provided by the applicant (e.g. 90-day toxicity studies in rodents or feeding studies in young rapidly growing animal species).

No substantial modifications in the composition of the food/feed derived from cotton 281-24-236 × 3006-210-23 × MON 88913 (see Section 3.3.2), no indication of possible unintended effects and no interactions were identified. Therefore, according to EFSA (2006a), no animal studies on the food/feed derived from cotton 281-24-236 × 3006-210-23 × MON 88913 are required.

3.3.3.4. Allergenicity

For the allergenicity assessment a weight-of-evidence approach is followed, taking into account all of the information on the newly expressed proteins (EFSA, 2006a; Codex Alimentarius, 2009). In addition, when known functional aspects of the newly expressed protein or structural similarity to known adjuvants may indicate an adjuvant activity, the possible role of these proteins as adjuvants is considered. When newly expressed proteins with a potential adjuvant activity are expressed together, possible interactions, increasing adjuvanticity and impacting the allergenicity of the GM crop are assessed.

Assessment of allergenicity of the newly expressed proteins

For allergenicity, the GMO Panel has previously evaluated the safety of the Cry1Ac, Cry1F, PAT and CP4 EPSPS proteins, and no concerns on allergenicity were identified in the context of the applications assessed (EFSA GMO Panel, 2010a, 2013). No new information on allergenicity of these proteins that might change the previous conclusions of the GMO Panel has become available. Based on current knowledge, and as none of the newly expressed proteins showed allergenicity, no reasons of concerns regarding the simultaneous presence of these newly expressed proteins in the three-event stack cotton affecting allergenicity were identified.

For adjuvanticity, proteins derived from *B. thuringiensis* (Bt proteins) have been suggested to possess adjuvant activity, based on animal studies on Cry1Ac when applied at relatively high doses (e.g. Vázquez et al., 1999). The Panel has previously evaluated the safety of the Cry1Ac and Cry1F proteins in the context of the two-event stack cotton 281-24-236 × 3006-210-23, and no concerns on adjuvanticity were identified (EFSA GMO Panel, 2010a, 2013). The levels of Bt proteins in the three-event stack cotton are similar to those in the parental lines (see Table 4). From the limited experimental evidence available, the GMO Panel did not find indications that the presence of the Bt proteins at the levels present in this three-event stack cotton might act as adjuvants with the potential to enhance a specific immunoglobulin E (IgE) response and to favour the development of an allergic reaction.

Assessment of allergenicity of the whole GM plant

Cotton is not considered to be a common allergenic food (OECD, 2009).[23] A few cases of food allergy to cottonseed have been reported (Atkins et al., 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 (see Sections 3.3.1 and 3.3.2),

[22] Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed (OJ L 14, 30.5.2002, p. 10).

[23] 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.

the GMO Panel identified no indications of a potentially increased allergenicity of the three-event stack cotton 281-24-236 × 3006-210-23 x MON 88913 food and feed with respect to non-GM cotton.

3.3.3.5. Nutritional assessment of GM food/feed

The intended trait of the three-event stack cotton 281-24-236 × 3006-210-23 x MON 88913 is herbicide tolerance and insecticide resistance, with no intention to alter the nutritional parameters. Comparison of the composition of the three-event stack cotton 281-24-236 × 3006-210-23 x MON 88913 with its conventional counterpart did not identify differences that would require a nutritional assessment as regards food and feed (see Section 3.3.2). From these data, the nutritional characteristics of the three-event stack cotton 281-24-236 × 3006-210-23 x MON 88913-derived food and feed are not expected to differ from those of its conventional counterpart.

3.3.3.6. Conclusion

In the context of this application, the GMO Panel considers that the newly expressed proteins do not raise safety concerns for human and animal health in the three-event stack cotton 281-24-236 × 3006-210-23 x MON 88913.

No adverse effects for human and animals resulting from interactions between the proteins are expected based on their known mode of action. The GMO Panel did not identify safety concerns regarding allergenicity or adjuvanticity of newly expressed proteins in this three-event stack cotton, or regarding the overall allergenicity of the three-event stack cotton. As relevant compositional differences were not observed between cotton 281-24-236 × 3006-210-23 x MON 88913 and its conventional counterpart, the nutritional value of food and feed derived from cotton 281-24-236 × 3006-210-23 x MON 88913 is not expected to differ from that of food and feed derived from its conventional counterpart.

3.3.4. Environmental risk assessment

3.3.4.1. Evaluation of relevant scientific data

Considering the scope of application EFSA-GMO-NL-2009-68, the ERA is concerned mainly 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; and (2) the accidental release into the environment of viable cottonseeds 281-24-236 × 3006-210-23 × MON 88913 during transportation and processing.

As the scope of the present application excludes cultivation, environmental concerns in the EU related to the use of glyphosate-based and glufosinate-ammonium-based herbicides on cotton 281-24-236 × 3006-210-23 × MON 88913 do not apply.

3.3.4.2. Environmental risk assessment[24]

Potential unintended effects on plant fitness due to genetic modification[25]

In Southern Europe, *G. herbaceum* and *G. hirsutum* have been grown since the 19th century, and led to transient or locally naturalised cotton plants in these areas (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, susceptibility to diseases and their low competitiveness (Eastick and Hearnden, 2006). For example, after the end of cotton cultivation in Italy in the 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).

Cotton 281-24-236 × 3006-210-23 x MON 88913 has been developed for protection against certain lepidopteran pests (e.g. cotton bollworm and tobacco budworm larvae) and tolerance to glyphosate- and glufosinate-ammonium based herbicides (the latter is primarily used as a selective marker). The increased resistance against cotton insect pests due to the expression of the *cry1Ac* and *cry1F* genes

[24] Dossier: Part I – Section D8-10 and Annex 2.
[25] Dossier: Part I – Sections D 7.1, 7.2, 7.4, 9.1, 9.2 and Study 050006.03.

may provide a selective advantage in situations where plant survival is affected by pest pressure. Also, the *CP4 epsps* and *pat* genes coding for a herbicide tolerance trait can provide a potential agronomic and selective advantage for this GM cotton plant when glyphosate- or glufosinate-based herbicides are applied.

Considering the scope of application EFSA-GMO-NL-2009-68, special attention is paid to those agronomic and phenotypic characteristics (for further details, see Section 3.3.2) which may be indicative of changes in the survival of cottonseeds 281-24-236 × 3006-210-23 × MON 88913 accidentally released into the environment, as well as in the establishment and fitness of GM cotton plants. As described in Section 3.3.2, GM cotton treated with conventional herbicides differed from its conventional counterpart in total nodes at 14 days, and node of first white flower at 42 days, trash (leaf) and fibre elongation (the latter also differed when treated with the intended herbicide). Given the biology of cotton, the differences observed in agronomic and phenotypic characteristics are unlikely to be biologically relevant in terms of increased fitness potential.

In the case of accidental release into the environment of cotton 281-24-236 × 3006-210-23 × MON 88913, there are no indications of an increased likelihood of establishment and spread of feral cotton 281-24-236 × 3006-210-23 × MON 88913 plants. Should these plants be exposed to glyphosate- and/or glufosinate-ammonium-based herbicides they are likely to exhibit a selective advantage that could increase their transient local occurrence. However, 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 cotton 281-24-236 × 3006-210-23 × MON 88913 in regions where it is cultivated and any change in survival capacity, including overwintering.

The GMO Panel concludes that it is very unlikely that cotton 281-24-236 × 3006-210-23 × MON 88913 will differ from conventional cotton varieties in its ability to survive or establish feral populations under European environmental conditions.

1) Potential for gene transfer[26]

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

a) Plant-to-bacteria gene transfer

The potential for horizontal gene transfer of the recombinant DNA of the three single events to bacteria was assessed in previous opinions (EFSA GMO Panel 2010a, 2013). No concern as a result of an unlikely, but theoretically possible, horizontal gene transfer of the recombinant genes to bacteria in the gut or other receiving environments was identified. Synergistic effects of the recombinant genes in increasing the likelihood for horizontal gene transfer, for instance because of combinations of recombinogenic sequences, were not identified. Therefore, the GMO Panel concludes that, considering the scope of this application, the unlikely, but theoretically possible, horizontal transfer of recombinant genes from cotton 281-24-236 × 3006-210-23 × MON 88913 to bacteria does not raise any environmental safety concern.

b) Plant-to-plant gene transfer

Considering the scope of the application EFSA-GMO-NL-2009-68 and the biology of cotton, a possible pathway to harm pertains to the potential of occasional feral GM cotton plants originating from accidental spillage of imported cottonseeds 281-24-236 × 3006-210-23 × MON 88913 to transfer recombinant DNA to sexually cross-compatible plants. Cotton is predominantly an annual self-pollinating crop, though cross-pollination can occur at low frequencies in the presence of insect pollinators (such as wild bees, honeybees and 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, any vertical gene transfer is limited to cultivated and feral cotton plants (no wild relatives of cotton have been reported in Europe). 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

[26] Dossier: Part I – Section D 9.3.

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 the cotton 281-24-236 × 3006-210-23 × MON 88913 in Europe will not differ from that of conventional cotton, even after exposure to glyphosate- or glufosinate-ammonium-based herbicides.

2) Interactions of the GM plant with target organisms[27]

Interactions between Cry1F and Cry1Ac might occur depending on the target species tested (Chakrabarti et al., 1998; Adamczyk and Gore, 2004). Considering the scope of application EFSA-GMO-NL-2009-68, and the low level of exposure of the environment to cotton 281-24-236 × 3006-210-23 × MON 889133, interactions of the GM cotton seeds or plants arising from spillage of imported seeds with target organisms are not considered a relevant issue by the GMO Panel, regardless of potential synergistic interactions that might occur between the different Cry proteins.

3) Interactions of the GM plant with non-target organisms[28]

Interactions between Cry1F and Cry1Ac, leading to synergistic insecticidal effects, might occur in other susceptible non-target species. However, considering the scope of application EFSA-GMO-NL-2009-68, and the low level of exposure of the environment to cotton 281-24-236 × 3006-210-23 × MON 88913 from spillage of imported seeds with non-target organisms are not considered a relevant issue by the GMO Panel.

The GMO Panel evaluated whether the expressed Cry1Ac and Cry1F proteins might potentially affect non-target organisms by entering the environment through faecal material of animals fed GM cotton seed products. Cry proteins are degraded by enzymatic activity in the gastrointestinal tract, meaning that only low amounts of intact Cry proteins would remain in the faeces. This was demonstrated for Cry1Ab (Einspanier et al., 2004; Lutz et al., 2005, 2006; Wiedemann et al., 2006; Guertler et al., 2008; Paul et al., 2010). Further degradation of the protein in the manure and faeces will take place because of microbiological proteolytic activity. In addition, there will be further degradation of Cry proteins in soil, reducing the possibility for exposure of potentially sensitive non-target organisms. Although Cry proteins may bind to clay minerals and organic 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 (Gruber et al., 2011; Valldor et al., 2015). The GMO Panel is not aware of evidence of released Cry proteins from GM plants causing significant negative effects on soil microorganisms.

Considering the scope of the application, it can be concluded that the exposure of potentially sensitive non-target organisms to the Cry1Ac and Cry1F proteins is likely to be very low and of no biological relevance, regardless of potential synergistic interactions that might occur between the different Cry proteins.

4) Interactions with the abiotic environment and biogeochemical cycles[29]

Considering the scope of application EFSA-GMO-NL-2009-68, and the low level of exposure to the environment, potential interactions with the abiotic environment and biogeochemical cycles were not considered a relevant issue by the GMO Panel.

3.3.4.3. Conclusion

No safety concerns with regard to the environment from the import and processing of cotton 281-24-236 × 3006-210-23 x MON 88913 were identified. There are no indications of an increased likelihood of the establishment and spread of occasional feral cotton 281-24-236 × 3006-210-23 x MON 88913 plants in the case of accidental release into the environment of viable GM cotton seeds even when exposed to the herbicides to which it has tolerance. The unlikely, but theoretically possible, horizontal transfer of recombinant genes from cotton 281-24-236 × 3006-210-23 x MON 88913 to

[27] Dossier: Part I – Section D 9.4.
[28] Dossier: Part I – Section D 9.5.
[29] Dossier: Part I – Section D 9.8.

bacteria does not raise any environmental safety concern. Considering the scope of the application, potential interactions of cotton 281-24-236 × 3006-210-23 × MON 88913 with the biotic and abiotic environment were not considered a relevant issue by the GMO Panel.

3.4. Post-market monitoring

3.4.1. Post-market monitoring of GM food/feed

The GMO Panel considers that post-market monitoring of food/feed derived from cotton 281-24-236 × 3006-210-23 × MON 88913 is not necessary, given the absence of safety concerns identified.

3.4.2. Post-market environmental monitoring[30]

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, 2006b; EFSA GMO Panel, 2011b). In addition, the GMO Panel acknowledges the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure in the case of accidental release of viable GM cottonseeds.

The PMEM plan proposed by the applicant includes (1) the description of a monitoring approach involving operators (federations involved in cotton import and processing) reporting any observed adverse effect(s) of the GMO on human health and the environment to the applicant, via a centralised system; (2) a coordinating system established by EuropaBio for the collection of information recorded by 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 period.

The GMO Panel is of the opinion that the PMEM plan proposed by the applicant is in line with the scope of application EFSA-GMO-NL-2009-68. As no potential adverse environmental effects were identified, case-specific monitoring was not considered necessary. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

4. Overall conclusions

No new data on the single cotton events 281-24-236, 3006-210-23 and MON 88913 that would lead to a modification of the original conclusions on their safety were identified.

The combination of cotton events 281-24-236, 3006-210-23 and MON 88913 in the three-event stack cotton did not give rise to issues – based on the molecular, agronomic, phenotypic or compositional characteristics – regarding food and feed safety and nutrition.

The combination of the newly expressed proteins in the three-event stack cotton did not raise concerns for human and animal health.

Considering the introduced traits and the outcome of the comparative analysis, the routes of exposure and the limited exposure levels, the GMO Panel concluded that this three-event stack cotton would not raise safety concerns in case of accidental release of viable GM cottonseeds into the environment.

Post-market monitoring of food/feed derived from the three-event stack cotton is not considered necessary.

The GMO Panel is of the opinion that the PMEM plans provided by the applicant are in line with the scope of the three-event stack cotton.

The GMO Panel concludes that the three-event stack cotton 281-24-236 × 3006-210-23 × MON 88913 is as safe and as nutritious as its conventional counterpart in the context of its scope.

[30] Dossier: Part I – Section D 11 and Annex 3.

Documentation requested and provided to EFSA

1) Letter from the Competent Authority of the Netherlands received on 19 March 2009 concerning a request for the authorisation for the placing on the market of genetically modified cotton 281-24-236 × 3006-210-23 × MON 88913 in accordance with Regulation (EC) No 1829/2003 submitted by Agrigenetics c/o Dow AgroSciences LLC (reference EFSA-GMO-NL-2009-68).
2) Acknowledgement letter dated 2 April 2009 from EFSA to the Netherlands Competent Authority.
3) Letter from EFSA to applicant dated 28 April 2009 requesting additional information under completeness check.
4) Letter from applicant to EFSA received on 17 May 2010 providing additional information under completeness check.
5) Letter from EFSA to applicant dated 4 June 2010 requesting additional information under completeness check.
6) Letter from applicant to EFSA received on 2 December 2010 providing additional information under completeness check.
7) Letter from EFSA to applicant dated 17 January 2011 requesting additional information under completeness check.
8) Letter from applicant to EFSA received on 9 February 2011 providing additional information under completeness check.
9) Letter from EFSA to applicant dated 8 December 2010 regarding the assessment of applications submitted under Regulation (EC) No 1829/2003 containing stacked events.
10) Letter from EFSA to applicant dated 3 March 2011 delivering the 'Statement of Validity' for application EFSA-GMO-NL-2009-68 (cotton 281-24-236 x 3006-210-23 x MON 88913) submitted by Agrigenetics c/o Dow AgroSciences LLC under Regulation (EC) No 1829/2003.
11) Letter from EFSA to applicant dated 4 March 2011 stopping the clock due to the pending assessment of the single event cotton MON 88913 (application EFSA-GMO-UK-2007-41).
12) Letter from EFSA to applicant dated 28 September 2012 requesting additional information and stopping the clock on behalf of the EURL-GMFF.
13) Letter from EFSA to applicant dated 5 August 2013 re-starting the clock due to the adoption of the Scientific Opinion on single event cotton MON 88913 (application EFSA-GMO-UK-2007-41) and maintaining the clock stopped due to EURL-GMFF questions.
14) Letter from EFSA to applicant dated 9 January 2014 re-starting the clock on behalf of the EURL-GMFF.
15) Letter from EFSA to applicant dated 17 January 2014 requesting additional information and stopping the clock.
16) Letter from applicant to EFSA received on 22 September 2014 providing additional information.
17) Letter from EFSA to applicant dated 31 October 2014 requesting additional information and maintaining the clock stopped.
18) Letter from applicant to EFSA received on 16 December 2014 providing additional information.
19) Letter from EFSA to applicant dated 29 January 2015 re-starting the clock.
20) Letter from applicant to EFSA received on 24 February 2015 providing additional information spontaneously.
21) Letter from applicant to EFSA received 2 March 2015 providing additional information spontaneously.
22) Letter from EFSA to applicant dated 25 March 2015 requesting additional information and stopping the clock.
23) Letter from applicant to EFSA received on 12 June 2015 providing additional information spontaneously.
24) Letter from EFSA to applicant dated 9 September 2015 re-starting the clock.
25) Letter from EFSA to applicant dated 14 October 2015 requesting additional information and stopping the clock.
26) Letter from EFSA to applicant dated 25 November 2015 requesting additional information and maintaining the clock stopped.
27) Letter from applicant to EFSA received 11 December 2015 providing additional information.
28) Letter from applicant to EFSA received on 26 January 2016 providing additional information.
29) Letter from applicant to EFSA received on 16 February 2016 providing additional information.
30) Letter from EFSA to applicant dated 29 February 2016 re-starting the clock.

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Abbreviations

ADF	acid detergent fibre
+b	colour (yellowness)
CTP2	Chloroplast transit peptide 2
EC	European Commission
ELISA	enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	environmental risk assessment
EURL-GMFF	European Union Reference Laboratory for GM Food & Feed
FA	fatty acid
FMV	figwort mosaic virus
GM	genetically modified
GMO	genetically modified organism
IgE	immunoglobulin E
NDF	neutral detergent fibre

OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PAT	phosphinothricin acetyltransferase
PMEM	post-market environmental monitoring
Rd	colour (reflectance)
UTR	untranslated region