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SCIENTIFIC OPINION

Scientific Opinion on an application (EFSA-GMO-NL-2010-85) for the placing on the market of MON 87769 × MON 89788 soybean, genetically modified to contain stearidonic acid and be tolerant to glyphosate for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The EFSA GMO Panel previously assessed the two single events that are combined to produce soybean MON 87769 × MON 89788 and did not identify safety concerns. No new data on these single events, leading to a modification of the original conclusions on safety, were identified. The molecular, agronomic, phenotypic and compositional data on soybean MON 87769 × MON 89788 did not give rise to safety concerns. The Panel considers that there is no reason to expect interactions between the single events to impact on food and feed safety. There were no concerns regarding the potential toxicity or allergenicity of soybean MON 87769 × MON 89788, and no evidence that the genetic modification significantly changes the overall allergenicity. Because of the lack of data on dietary exposure to refined bleached deodorised oil from soybean MON 87769 × MON 89788, the EFSA GMO Panel could not complete the human health and nutrition assessment. There are no concerns regarding the use of feedingstuffs derived from defatted toasted MON 87769 × MON 89788 soybean meal. There are no indications of an increased likelihood of establishment and spread of feral soybean plants. Potential interactions of soybean MON 87769 × MON 89788 with biotic and abiotic environments were not considered relevant to this application. The unlikely, but theoretically possible, transfer of recombinant genes from soybean MON 87769 × MON 89788 to environmental bacteria is not of safety concern. The post-market environmental monitoring plan and reporting intervals conform with the scope of this application. In conclusion, the Panel could not complete the food and feed safety assessment of soybean MON 87769 × MON 89788 because of the lack of an appropriate nutritional assessment. The Panel concludes that soybean MON 87769 × MON 89788 is unlikely to have adverse effects on the environment in the context of application EFSA-GMO-NL-2010-85.

KEY WORDS

GMO, soybean (*Glycine max* (L.) Merr.), CP4 EPSPS, herbicide tolerant, production of stearidonic acid, stack

[1] On request from the Competent Authority of the Netherlands on an application (EFSA-GMO-NL-2010-85) submitted by Monsanto, Question No EFSA-Q-2010-01086, adopted on 17 September 2015.

[2] Panel members: Andrew Nicholas Birch, Josep Casacuberta, Adinda De Schrijver, Achim Gathmann, Mikolaj Antoni Gralak, Philippe Guerche, Huw Jones, Barbara Manachini, Antoine Messéan, Hanspeter Naegeli, Elsa Ebbesen Nielsen, Fabien Nogué, Christophe Robaglia, Nils Rostoks, Jeremy Sweet, Christoph Tebbe, Francesco Visioli and Jean-Michel Wal. Correspondence: gmo@efsa.europa.eu

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SUMMARY

Following the submission of application EFSA-GMO-NL-2010-85 under Regulation (EC) No 1829/2003 from Monsanto, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) was asked to deliver a scientific opinion on the safety of herbicide-tolerant, stearidonic acid (SDA)-producing genetically modified (GM) soybean MON 87769 × MON 89788 (Unique Identifier MON-87769-7 × MON-89788-1). The scope of application EFSA-GMO-NL-2010-85 is for food and feed uses, import and processing, but excludes cultivation within the European Union (EU).

Soybean containing the single events MON 87769 (expressing the Δ15 desaturase protein from *Neurospora crassa* (NcΔ15D) and the Δ6 desaturase protein from *Primula juliae* (PjΔ6D)) and MON 89788 (expressing the CP4 protein 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)) were assessed previously and no concerns were identified for human and animal health or environmental safety. No safety concern was identified by updated bioinformatic analyses, or reported by the applicant with regard to the two single soybean events, since the publication of the corresponding scientific opinions. Consequently, the EFSA GMO Panel considers that its previous conclusions on the safety of the single soybean events remain valid.

The two-event stack soybean MON 87769 × MON 89788 was produced by conventional crossing of the soybean lines MON 87769 and MON 89788, combining the production of SDA and the tolerance to glyphosate-based herbicides. The EFSA GMO Panel evaluated soybean MON 87769 × MON 89788 with reference to the scope and appropriate principles described in its guidelines for the risk assessment of GM plants and derived food and feed, the environmental risk assessment of GM plants and the post-market environmental monitoring (PMEM) of GM plants. The scientific evaluation of the risk assessment included molecular characterisation of the inserted DNA and analysis of the expression of the corresponding proteins. An evaluation of the comparative analyses of the compositional, agronomic and phenotypic characteristics was undertaken, and the safety of the newly expressed protein and the whole food/feed was evaluated with respect to potential toxicity, allergenicity and nutritional wholesomeness. Evaluations of environmental impacts and the PMEM plan were also undertaken. In accordance with the EFSA GMO Panel guidance document applicable to this application (EFSA GMO Panel, 2011a), "*For GM plants containing a combination of transformation events (stacked events) the primary concern for risk assessment is to establish that the combination of events is stable and that no interactions between the stacked events, that may raise safety concerns compared to the single events, occur. The risk assessment of GM plants containing stacked events focuses on issues related to: a) stability of the inserts, b) expression of the introduced genes and their products and c) potential synergistic or antagonistic effects resulting from the combination of the events*".

The molecular data establish that the transformation events stacked in soybean MON 87769 × MON 89788 have the same molecular properties and characteristics as the single transformation events. Comparison of the levels of the NcΔ15D, PjΔ6D and CP4 EPSPS proteins between the stack and the corresponding single events did not reveal an interaction that would affect protein or trait expression levels in a way that would give rise to safety concerns. The biological functions of the newly expressed proteins did not suggest the possibility of interactions between the events at a functional level.

The EFSA GMO Panel considered the compositional, phenotypic and agronomic data supplied and the observed statistically significant differences between soybean MON 87769 × MON 89788 and its comparator, in the light of the field trial design, measured biological variation and the level of the studied compounds in commercial non-GM soybean varieties. No relevant differences were identified in the compositional characteristics of soybean MON 87769 × MON 89788 in comparison with its comparator, except for the altered fatty acid composition (of SDA, γ-linolenic acid and two trans-fatty acids) and a reduction in linoleic acid.

The safety assessment identified no concerns regarding the potential toxicity and allergenicity of the newly expressed PjΔ6D, NcΔ15D and CP4 EPSPS proteins, and found no evidence that the genetic modification might significantly change the overall allergenicity of soybean MON 87769 × MON 89788. The EFSA GMO Panel could not complete a full assessment on the possible impact of MON 87769 × MON 89788 soybean oil on health and nutrition, because of the lack of data on dietary exposure to refined bleached deodorised (RBD) oil from MON 87769 × MON 89788 soybean. There are no concerns regarding the use of feeding stuffs derived from defatted toasted MON 87769 × MON 89788 soybean meal.

No safety concerns for the environment from the import and processing of soybean MON 87769 × MON 89788 were identified. There are no indications of an increased likelihood of establishment and spread of feral soybean MON 87769 × MON 89788 plants in the case of accidental release into the environment of viable GM soybean seeds. The unlikely, but theoretically possible, transfer of the recombinant genes from soybean MON 87769 × MON 89788 to bacteria does not give rise to a safety concern for these bacteria owing to the lack of a selective advantage. Potential interactions of soybean MON 87769 × MON 89788 with the biotic and abiotic environment were not considered relevant by the EFSA GMO Panel. The PMEM plan provided by the applicant and the reporting intervals are in line with the scope of application EFSA-GMO-NL-2010-85.

In conclusion, the EFSA GMO Panel could not complete the food and feed safety assessment of soybean MON 87769 × MON 89788 because of the lack of an appropriate nutritional assessment. The EFSA GMO Panel concludes that soybean MON 87769 × MON 89788 is unlikely to have any adverse effects on the environment, considering the scope of application EFSA-GMO-NL-2010-85.

As a full assessment on the possible health and nutritional impact of MON 87769 × MON 89788 soybean oil was not made, the EFSA GMO Panel is not in the position to comment on the post-market monitoring plan and labelling provided by the applicant, in accordance with Articles 13(2)(a) and 25(2)(c) of Regulation (EC) No 1829/2003.

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BACKGROUND

On 30 July 2010, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands application EFSA-GMO-NL-2010-85, for authorisation of genetically modified (GM) soybean MON 87769 × MON 89788 submitted by Monsanto within the framework of Regulation (EC) No 1829/2003 for food and feed uses, import and processing[4].

After receiving the application EFSA-GMO-NL-2010-85 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, and made the summary of the application available to the public on the EFSA website[5]. 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 5 November 2010, EFSA received additional information (requested on 9 September 2010). On 26 November 2010, 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 European Commission, and consulted nominated risk assessment bodies of Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC[6] following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Member States had three months after the date of receipt of the valid application (from 21 May 2014 to 21 August 2014)[7] to make their opinion known.

The EFSA GMO Panel carried out an evaluation of the scientific risk assessment of soybean MON 87769 × MON 89788 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. The EFSA 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, 2006), the environmental risk assessment of GM plants (EFSA GMO Panel, 2010) and on the post-market environmental monitoring of GM plants (EFSA GMO Panel, 2011b). Furthermore, the EFSA GMO Panel also took into consideration the scientific comments of Member States, the additional information provided by the applicant and the relevant scientific publications.

On 14 July 2014, 25 July 2014, 10 November 2014 and 30 March 2015, the EFSA GMO Panel requested additional information from the applicant. The applicant provided the requested information on 12 September 2014, 15 September 2014, 28 January 2015, 1 June 2015 and 10 July 2015. The applicant also spontaneously provided additional information on 14 October 2013.

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

According to Regulation (EC) No 1829/2003 (EC, 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).

[4] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.
[5] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2010-01086
[6] 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.
[7] Upon validation, application EFSA-GMO-NL-2010-85 was stopped pending the finalisation of application EFSA-GMO-NL-2008-76 (soybean MON 87769). The scientific opinion on application EFSA-GMO-NL-2008-76 was adopted on 10 April 2014.

TERMS OF REFERENCE

The EFSA GMO Panel was requested to carry out a scientific assessment of soybean MON 87769 × MON 89788 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 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.

Since the EFSA GMO Panel was not in the position to make a full assessment on possible health and nutritional impact of soybean MON 87769 × MON 89788, the need for a specific labelling in accordance with Articles 13(2) (a) and 25(2)(c) of Regulation (EC) No 1829/2003 was not considered. Neither did the EFSA GMO Panel consider methods of detection (including sampling and the identification of the specific transformation event in the food/feed and/or food/feed produced from it), which are matters related to risk management.

ASSESSMENT

1. Introduction

Application EFSA-GMO-NL-2010-85 covers the two-event stack soybean MON 87769 × MON 89788 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).

The European Food Safety Authority (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, 2006, 2007). Additional information, received after May 2011, was assessed in accordance with the EFSA 2011 guidance (EFSA GMO Panel, 2011a).

Soybean MON 87769 × MON 89788 was developed to produce stearidonic acid (SDA) and to confer tolerance to glyphosate (*N*-(phosphonomethyl)glycine)-based herbicides. The production of SDA is achieved by the expression of the Δ6 desaturase protein from *Primula juliae* (PjΔ6D) and the Δ15 desaturase protein from *Neurospora crassa* (NcΔ15D). Tolerance to glyphosate is achieved by expression of CP4 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS).

The two single soybean events MON 87769 and MON 89788 have been previously assessed (see Table 1) on the basis of experimental data. No concerns for human and animal health or environmental safety were identified.

Table 1: Single soybean events already assessed by the EFSA Panel on Genetically Modified Organisms

Event	Application	EFSA scientific opinion
MON 87769	EFSA-GMO-NL-2008-76	EFSA GMO Panel (2014)
MON 89788	EFSA-GMO-NL-2005-36	EFSA (2008)

2. Issues raised by Member States

Issues raised by Member States on soybean MON 87769 × MON 89788 were considered in this scientific opinion and are addressed in detail in Annex G of the EFSA overall opinion[8].

3. Updated information on single events

Since the publication of the scientific opinions on the single soybean events by the EFSA Panel on Genetically Modified Organisms (GMO) (EFSA, 2008; EFSA GMO Panel, 2014), no safety issues pertaining to the two single events have been reported by the applicant.

Updated bioinformatic analyses on the junction regions for events MON 87769 and MON 89788 confirmed that no known endogenous genes were disrupted by any of the inserts[9]. Updated bioinformatic analyses of the amino acid sequences of the newly expressed proteins and of the open reading frames (ORFs) in the inserts and spanning the junction regions revealed no new significant similarities to known toxins or allergens[10]. The similarity to allergens search used a criterion of 35 % identity to the amino acid sequence of known allergens in a window of 80 amino acids. No matches of eight contiguous identical amino acid sequences between these proteins and known allergens were found, with the exception of one match of eight contiguous serine residues (SSSSSSSS) which was already assessed by the EFSA GMO Panel (see Section 5.1.4.1 of EFSA GMO Panel, 2014).

[8] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2015-00551
[9] Additional information: 10/07/2015.
[10] Additional information: 10/07/2015.

Having assessed the updated information on soybean MON 87769 × MON 89788, the EFSA GMO Panel considers that its previous conclusions on the safety of the single soybean events remain valid.

4. Risk assessment of the two-event stack soybean MON 87769 × MON 89788

4.1. Molecular characterisation

The possible interactions between the known biological functions conferred by the individual inserts and interactions that would affect protein or trait expression level are considered.

4.1.1. Genetic elements and biological functions of the inserts[11]

Soybean MON 87769 and MON 89788 are combined by conventional crossing to produce soybean MON 87769 × MON 89788. The structure of the inserts introduced into soybean MON 87769 × MON 89788 is described in detail in previous EFSA scientific opinions (EFSA, 2008; EFSA GMO Panel, 2014), and no new genetic modifications were involved. The genetic elements in the expression cassettes of the single events are summarised in Table 2.

Table 2: Genetic elements in the expression cassettes of the events stacked in soybean MON 87769 × MON 89788

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
MON 87769	*7Sα′* from the *SphasI* gene (*Glycine max*)	*7Sα′* from the *SphasI* gene (*Glycine max*)	No	*Pj.D6D* (*Primula juliae*)	*Tml* (*Agrobacterium tumefaciens*)
	7Sα from the *Sphas2* gene (*Glycine max*)	*7Sα* from the *Sphas2* gene (*Glycine max*)	No	*Nc.Fad3* (*Neurospora crassa*)	*E9* (*Pisum sativum*)
MON 89788	*FMV/Tsf1* (*Arabidopsis thaliana*)	*Tsf1* (*Arabidopsis thaliana*)	*CTP2* (*Arabidopsis thaliana*)	*CP4 epsps* [a] (*Agrobacterium* sp. CP4)	*E9* (*Pisum sativum*)

a: Codon-optimised for expression in plants.
FMV, figwort mosaic virus; UTR, untranslated region.

There are three newly expressed proteins in soybean MON 87769 × MON 89788, all of which are enzymes. The biological functions conferred by these proteins are summarised in Table 3.

Table 3: Biological functions related to the events stacked in soybean MON 87769 × MON 89788

Event	Protein	Function in donor organism	Function in GM plant
MON 87769	NcΔ15D	Donor organism: *Neurospora crassa* Δ15 desaturase converts linoleic acid to α-linolenic acid (Stafford et al., 1998)	The Δ6 and Δ15 desaturases act together in the GM plant leading to the accumulation of stearidonic acid (Eckert et al., 2006; Vrinten et al., 2007; Haslam et al., 2013)
	PjΔ6D	Donor organism: *Primula juliae* Δ6 desaturase converts α-linolenic acid to stearidonic acid and can also convert linoleic acid to γ-linolenic acid (Sayannova et al., 2006; Ruiz-Lopez et al.,2009)	
MON 89788	CP4 EPSPS	Donor organism: *Agrobacterium* strain CP4. 5-Enolpyruvyl-shikimate-3-phosphate (EPSPS) synthase is an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Herrmann, 1995). Glyphosate is a competitive inhibitor of this enzyme	The bacterial CP4 EPSPS confers tolerance to glyphosate-based herbicides as it has a greatly reduced affinity towards glyphosate than the plant endogenous enzyme.

[11] Dossier: Part I—Section C.

4.1.2. Integrity of the events in the two-event stack soybean MON 87769 × MON 89788

The genetic stability of the inserted DNA over multiple generations in the single soybean events MON 87769 and MON 89788 was demonstrated previously (EFSA, 2008; EFSA GMO Panel, 2014). The integrity of these events in soybean MON 87769 × MON 89788 was demonstrated by Southern analyses[12] in the third self-pollinating generation after crossing the parental lines[13].

4.1.3. Information on the expression of the inserts[14]

Plants were grown at five locations (three replicate blocks) under field conditions in the USA in 2007. The levels of the PjΔ6D and NcΔ15D proteins in the two-event stack soybean and the single event MON 87769 were quantified by Western blot, while the levels of CP4 EPSPS were analysed by enzyme-linked immunosorbent assay (ELISA) in the two-event stack soybean and the single event MON 89788. Protein levels were determined in over season leaf (OSL1, OSL2, OSL3 and OSL4), forage, root, mature and immature seed. The data on mature seeds are reported and discussed below (Table 4). PjΔ6D, NcΔ15D and CP4 EPSPS protein levels in the two-event stack soybean were similar to the corresponding levels in the single-event soybean plants.

Table 4: Means, standard deviations and ranges (n = 15) of protein levels in mature seeds (µg/g dry weight) from soybean MON 87769, MON 89788 and the two-event stack soybean

Event / Protein	MON 87769 × MON 89788	MON 87769	MON 89788
PjΔ6D	3.4[a] ± 2.3[b] 0.76-10[c]	3.0 ± 3.3 0.69-9.2	---
NcΔ15D	9.6 ± 3.2 3.4-16	8.7 ± 3.8 3.4-17	---
CP4 EPSPS	120 ± 24 70-160	---	90 ± 31 33-140

a: mean
b: standard deviation
c: range
'---': not assayed

As the promoter used is a seed-specific promoter and, in the single events, the expression in immature seeds was shown to be markedly higher than in mature seeds, the PjΔ6D and NcΔ15D levels were also analysed in two-event stack immature soybean seeds. The mean levels of PjΔ6D were ca. 46 ± 32 µg/g dry weight (dw) with a range of 13–130 µg/g dw for immature seeds. The mean NcΔ15D levels were ca. 120 ± 60 µg/g dw with a range of 33–290 µg/g dw for immature seeds. As previously observed for the single events, the levels for the two proteins were shown to be higher in immature than in the mature seeds.

4.1.4. Conclusion with regard to the molecular characterisation

The molecular data establish that the transformation events stacked in soybean MON 87769 × MON 89788 have the same molecular properties and characteristics as the single transformation events. The comparison of the NcΔ15D, PjΔ6D and CP4 EPSPS protein levels between the two-event stack soybean and the single events did not reveal an interaction that would affect protein or trait expression level in a way that would require further assessment. The biological functions of the newly expressed proteins do not suggest the possibility of interactions between the events at the functional level (see Section 4.3.2.1).

[12] Dossier: Part I—Section D2(a).
[13] Dossier: Part I – Section D3.
[14] Dossier: Part I—Section D3.

4.2. Comparative analysis

4.2.1. Evaluation of relevant scientific data

4.2.1.1. Choice of comparator and production of material for the comparative analysis[15]

In application EFSA-GMO-NL-2010-85, the applicant supplied data on agronomic and phenotypic characteristics of soybean MON 87769 × MON 89788 from a set of field trials carried out at five locations in the major soybean growing regions of Argentina during the 2007/2008 season[16]. A maintenance regime based on conventional herbicides was applied to all materials. This experimental design allows a direct comparison between the double-event stack soybean and its comparator treated under the same management regimes (including conventional herbicides). The treatment of the genetically modified (GM) soybean with glyphosate-based herbicides, which would have allowed the assessment of herbicide effects, was not included.

The applicant supplied data on the composition of forage and seeds of soybean MON 87769 × MON 89788 and its comparator harvested from another set of field trials carried out at five locations in the major soybean growing regions of the USA in 2007[17]. While the comparator A3525 and the non-GM soybean varieties received only conventional herbicide treatment ("untreated"), soybean MON 87769 × MON 89788 received a single application of a glyphosate-based herbicide (between growth stage V2 to R1) in addition to the conventional herbicide treatment ("treated").

In both sets of field trials, the test materials were grown in a randomised complete block design with three replicates. Because the GM events in soybean MON 87769 × MON 89788 were introduced into the Asgrow A3525 genetic background, the comparator used was the Asgrow soybean variety A3525. Each block at each of the field trial sites included soybean MON 87769 × MON 89788, the comparator A3525, and three to four commercial non-GM soybean varieties[18]. In total, 12 non-GM soybean varieties, with similar maturity classifications, were included across field trial sites in Argentina[19], and 15 varieties were included across the field trial sites in the USA[20].

The test materials soybean MON 87769 × MON 89788 and A3525 soybean were characterised by event-specific polymerase chain reactions (PCRs) for the presence or absence of the MON 87769 and MON 89788 events. These studies confirmed an adequate quality of the test materials. The identity of the commercial non-GM soybean reference varieties were confirmed by chain-of-custody documentation.

Data on compositional, agronomic and phenotypic endpoints were statistically analysed for potential differences between soybean MON 87769 × MON 89788 and A3525 soybean using two analysis of variance (ANOVA) models: an across-site ANOVA (all trial sites combined) followed by an

[15] Technical dossier/Sections A3.1–3.2 and additional information received on 2/09/2013 and 20/06/2014.
[16] Alejo Ledesma, Córdoba; San Francisco, Santa Fe; Tacuari, Buenos Aires; Gahan, Buenos Aires; and Inès Indart, Buenos Aires.
[17] Jefferson County, Iowa (IA); Ottawa County, Michigan (MI); York County, Nebraska (NE); Berks County, Pennsylvania (PA); and Walworth County, Wisconsin (WI).
[18] Four commercial non-GM soybean reference varieties were included at each field trial site in Argentina in the season 2007/2008, and three at each field trial site in the USA in the season 2007.
[19] The commercial non-GM soybean reference varieties included in the field trials in Argentina were Asgrow A3244, Lewis 372, CB3461, Quality Plus, Hoegemeyer 333, Croplan 3596STS, NK 32Z3, Garst 3585N, Stine 3300-0, Stewart 3454 and Pioneer 93B52.
[20] The commercial non-GM soybean reference varieties included in the field trials in the USA were Asgrow A3244, A2869, ST 3870-0, CB 3461, CB 37002, NK32Z3, Garst 3585N, Stine 3300-0, Stine 2788, Stine 3608-0, Pioneer 93B52, QP 365C, HT 3596STS and MG-M3444.

individual-site analysis[21]. No statistical comparisons were made between soybean MON 87769 × MON 89788 and the set of non-GM soybean commercial varieties.

4.2.1.2. Agronomic and phenotypic characteristics[22]

The phenotypic and agronomic characteristics evaluated at the five field trial sites in Argentina were early stand count, seedling vigour, plant growth stages, days to 50 % flowering, flower colour, plant pubescence, plant height, final stand count, lodging, pod shattering, seed moisture, 100 seed weight, test weight, yield, plant response to abiotic stressors, and plant response to disease damage.

The ANOVA across field trial sites showed a significant difference between soybean MON 87769 × MON 89788 and its comparator in mean plant height (59.8 ± 2.74 cm in soybean MON 87769 × MON 89788 vs. 57.2 ± 2.32 cm in the comparator). If field trial sites were analysed separately, the difference was observed at only one site. The range of the mean plant height observed for the commercial non-GM soybean reference varieties was 45.2–67.8 cm. The observed mean for soybean MON 87769 × MON 89788 falls within the range of commercial varieties.

Three site-specific abiotic stressors[23] and three diseases were evaluated on a continuous 0–9 symptom scale by experienced field coordinators four times during the growing season. Observations were considered to be different between soybean MON 87769 × MON 89788 and its comparator at a particular day and site if the scores did not overlap. No differences in response to abiotic stress were noted in any of the 60 comparisons. There were also no differences in response to disease damage in 58 of 60 comparisons. A difference was observed for two diseases; one at each of two sites at one observation. Finally, there were three statistically significant differences in arthropod damage detected out of 85 comparisons between soybean MON 87769 × MON 89788 and its comparator in the individual-site analysis but there were no overall differences in arthropod damage in any of the 24 comparisons.

4.2.1.3. Compositional analysis[24]

Soybean forage and seeds of soybean MON 87769 × MON 89788, its comparator and the commercial non-GM varieties harvested from the field trials carried out in the USA during the 2007 growing season were analysed for 75 constituents (68 in seeds[25] and 7 in forage[26]), including the key constituents recommended by the Organisation for Economic Co-operation and Development (OECD, 2001). Twenty-six parameters that had 50 % or more sample values below the assay limit of

[21] In both models, the overall mean and the genotype effect were fixed factors. The random factors (apart from residual error) were the block effect for the individual-site analysis, the site effect, the block-within-site effect and the site-by-genotype interaction for the across-site analysis.
[22] Technical dossier/Section D4 and additional information received 28/01/2015 and 01/06/2015.
[23] Drought, flooding, hail, soil compaction, strong wind and temperature stress.
[24] Technical dossier/Section D7.1.
[25] Protein, total fat, ash, moisture, carbohydrate by calculation, acid detergent fibre (ADF), neutral detergent fibre (NDF), caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), myristoleic acid (C14:1), pentadecanoic acid (C15:0), pentadecenoic acid (C15:1), palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), heptadecenoic acid (C17:1), stearic acid (C18:0), oleic acid (C18:1), total trans C18:1, linoleic acid (C18:2), isolinoleic acid (C18:2), total trans C18:2, linolenic acid (C18:3), γ-linolenic acid (C18:3), trans-α-linolenic acid (C18:3), other trans C18:3, stearidonic acid (C18:4), trans-stearidonic acid (C18:4), arachidic acid (C20:0), eicosenoic acid (C20:1), eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3), arachidonic acid (C20:4), EPA (eicosapentaenoic acid; C20:5), behenic acid (C22:0), erucic acid (C22:1), DPA (docosapentaenoic acid, C22:5), DHA (docosahexaenoic acid, C22:6), lignoceric acid (C24:0), methionine, cystine, tryptophan, threonine, isoleucine, histidine, valine, leucine, arginine, phenylalanine, lysine, glycine, alanine, aspartic acid, glutamic acid, proline, serine, tyrosine, daidzein, glycitein, genistein, stachyose, raffinose, lectin, phytic acid and trypsin inhibitor.
[26] Moisture, crude protein, crude fat, ash, crude fibre, acid detergent fiber (ADF), neutral detergent fiber (NDF) and total carbohydrate by calculation.

quantitation were excluded from the statistical analysis[27]. Four of these (γ-linolenic acid, SA, trans-α-linolenic acid (trans-ALA), trans-SA) occurred at quantifiable levels in seed of soybean MON 87769 × MON 89788, but at levels below the limit of quantitation in soybean A3525.

The across-site statistical analysis using a mixed model ANOVA of compositional data on soybean forage identified significant differences in only the level of moisture and total fat between soybean MON 87769 × MON 89788 and its comparator. As shown in Table 5, the identified levels for moisture content and total fat in soybean MON 87769 × MON 89788 were within the variation observed in commercial non-GM soybeans. The EFSA GMO Panel considered that none of the statistical differences in forage constituents was of relevance or needs further assessment.

Table 5: Constituents (least square mean) occurring at significantly different levels in forage and seeds of soybean MON 87769 × MON 89788 and its comparator A3525, harvested from field trials in the USA in 2007

Constituents	Estimated means across locations		
	MON 87769 × MON 89788 ("treated")	Comparator A3525 ("untreated")	Observed ranges of variation of non-GM soybean reference varieties ("untreated")
Forage			
Moisture (% fresh weight)	73.27	73.98	69.90–79.90
Total fat (% dw)	6.97	6.40	2.67–9.59
Seeds			
Palmitic acid (C16:0) (% total fatty acids)	12.32	11.80	9.91–12.15
Stearic acid (C18:0) (% total fatty acids)	4.22	4.12	3.61–4.93
Oleic acid (C18:1) (% total fatty acids)	18.10	20.37	19.17–26.06
Linoleic acid (C18:2) (% total fatty acids)	25.42	54.25	51.08–58.44
Linolenic acid (C18:3) (% total fatty acids)	10.70	8.68	7.24–8.50
Arachidic acid (C20:0) (% total fatty acids)	0.34	0.31	0.25–0.36
Eicosenoic acid (C20:1) (% total fatty acids)	0.18	0.16	0.15–0.19
Behenic acid (C22:0) (% total fatty acids)	0.28	0.30	0.29–0.38
Carbohydrates (% dw)	34.57	37.37	32.41–39.15
Protein (% dw)	41.84	40.70	38.01–43.18
Total fat (% dw)	17.95	16.38	16.79–21.92
Arginine (% total protein)	8.56	8.34	7.39–8.42
α-Tocopherol (mg/100 g dw)	2.15	1.94	1.05–2.75
Phytic acid (% dw)	1.34	1.24	0.92–1.69
Daidzein (μg/g dw)	1040.47	1477.34	540.83–1429.49
Genistein (μg/g dw)	705.74	991.32	637.53–1642.84
SDA (% total fatty acid)	21.62	–	–
Trans-SDA (% total fatty acid)	0.14	–	–
γ–Linolenic acid (% total fatty acid)	6.49	–	–
Trans-ALA (% total fatty acid)	0.20	–	–

–: Below the limit of quantification.

[27] The parameters excluded from the statistical analysis were caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), myristoleic acid (C14:1), pentadecanoic acid (C15:0), pentadecenoic acid (C15:1), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), heptadecenoic acid (C17:1), total trans C18:1, isolinoleic acid (C18:2), total trans C18:2, γ-linolenic acid (C18:3), trans-α-linolenic acid (C18:3), other trans C18:3, stearidonic acid (C18:4), trans-stearidonic acid (C18:4), eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3), arachidonic acid (C20:4), EPA (eicosapentaenoic acid; C20:5), erucic acid (C22:1), DPA (docosapentaenoic acid, C22:5), DHA (docosahexaenoic acid, C22:6) and lignoceric acid (C24:0).

As expected, owing to the genetic modification characterising the event MON 87769, significant differences in seed fatty acid composition were observed between soybean MON 87769 × MON 89788 and its comparator (Table 5). The altered fatty acid profile was accompanied by a slight increase in total fat content of the seed, but it remained within the range characterising the commercial non-GM soybean varieties analysed in the study.

The reductions in linoleic acid and in oleic acid were accompanied by the appearance of two metabolites: SDA (21.6%) and γ-linolenic acid (GLA) (6.5%). In addition, low amounts of two trans-fatty acids not occurring at measurable concentrations in commercial soybean oil were detected. These trans-fatty acids were 9c,12c,15t trans-ALA (18:3), at 0.20 % of total fatty acids, and 6c,9c,12c,15t trans-SDA (C18:4), at 0.14 % of total fatty acids. These major alterations in the fatty acid profile of the fat portion of seeds of soybean MON 87769 × MON 89788 were accompanied by altered levels of several other fatty acids (an increase in the proportion of palmitic acid, stearic acid, linolenic acid, arachidic acid and eicosenoic acid, and a decrease in the proportion of behenic acid and linoleic acid). Except for linoleic and linolenic acid, the levels observed in soybean MON 87769 × MON 89788 were within the variability of these constituents in conventional soybean varieties. The change in the levels of these fatty acids in the GM soybean would have no nutritional consequences and therefore are of no relevance for food and feed safety. The levels of linolenic acid observed in soybean MON 87769 × MON 89788 were within the range reported in the literature (Padgette et al., 1996) and the EFSA GMO Panel considered that the increase in linolenic acid did not need further assessment for food and feed safety.

The statistical analysis also revealed an increase in the protein content and a reduction in the carbohydrate content of seeds. As the carbohydrate content is calculated by taking the difference from the sum of the other proximate constituents, the apparent reduction of this parameter is likely to be a consequence of the altered protein and total fat content. The levels of both constituents of soybean MON 87769 × MON 89788 fell within the range established by the commercial non-GM soybean varieties analysed in the study. Although the arginine level in soybean MON 87769 × MON 89788 treated with the intended herbicide was outside the range of the non-GM soybean reference varieties, the EFSA GMO Panel concluded that no further assessment was needed as the reported differences would have no nutritional consequences and are not relevant to food and feed safety. A reduction in daidzein and genistein content of about 30 % was observed. However, because of the characteristic variability in isoflavone levels in soybean, the isoflavone levels were still within the range of the commercial non-GM soybean varieties included in the field trials.

4.2.2. Conclusion

The EFSA GMO Panel confirms that soybean MON 87769 × MON 89788 differs from its comparator and other non-GM soybean reference varieties by having an altered fatty acid profile and a higher level of SDA, as addressed in Section 4.3. None of the other differences identified in the composition of grain and forage obtained from soybean MON 87769 × MON 89788 requires further assessment with regard to food and feed safety.

The difference in plant height between soybean MON 87769 × MON 89788 and the comparator is further assessed for its potential environmental impact in Section 4.4.

4.3. Food and feed safety assessment

4.3.1. Effect of processing[28]

Soybean MON 87699 × MON 89788 will undergo the existing methods of production and processing used for commercial soybean. No novel method of production and processing is envisaged.

[28] Additional information: 03/06/2015.

Seeds of soybean MON 87769 × MON 89788 collected from the 2007 USA field trials were processed into refined bleached deodorised (RBD) oil and analysed for fatty acid composition. The applicant indicated that the intended effects of the genetic modification on the fatty acid pattern already seen in the analysis of unprocessed soybean seeds were also reflected in the composition of RBD oil obtained from soybean MON 87769 × MON 89788 (Table 6).

Table 6: Fatty acid composition of RBD oil and seeds of soybean MON 87769 × MON 89788 based on two composite samples analysed

Fatty acid	MON 87769 × MON 89788 RBD oil, mean (% total FA)	MON 87769 × MON 89788 Unprocessed seed, mean (% total FA)
16:0 Palmitic acid	12.36	12.32
18:0 Stearic acid	4.27	4.22
18:1 Oleic acid	18.10	18.10
18:2 Linoleic acid	25.28	25.42
18:3 GLA	6.45	6.49
18:3 Linolenic acid	10.56	10.70
18:3 trans-ALA	0.29	0.20
18:4 SDA	21.38	21.62
18:4 trans-SDA	0.24	0.14
20:0 Arachidic acid	0.35	0.34
20:1 Eicosenoic acid	0.23	0.18
22:0 Behenic acid	0.29	0.28

FA, fatty acid.

The influence of the modified fatty acid pattern seen in the unprocessed soybean seeds on the various products obtained after seed processing was described and assessed by the EFSA GMO Panel for soybean MON 87769 (EFSA GMO Panel, 2014). The products studied included RBD oil, isolated soy protein, toasted defatted meal and crude lecithin.

As observed for MON 87769, the modified fatty acid composition of soybean MON 87769 × MON 89788 seeds is also reflected in the composition of the RBD oil.

The oil of soybean MON 87769 × MON 89788 has a fatty acid profile that is more similar to other types of vegetable oil (e.g. olive oil) than oil from conventional soybean. Therefore, the production of food-quality oil from soybean MON 87769 × MON 89788 (as from MON 87769) is expected to be kept separate from the production of oil from conventional soybean varieties.

4.3.2. Toxicology

4.3.2.1. Toxicological assessment of newly expressed proteins

The newly expressed proteins in soybean MON 87769 × MON 89788 are the desaturases PjΔ6D and NcΔ15D, and the CP4 EPSPS protein.

All of these have been assessed in the context of the corresponding single events (PjΔ6D and NcΔ15D in MON 87769 (EFSA GMO Panel, 2014) and CP4 EPSPS in MON 89788 (EFSA, 2008)) and no safety concerns for humans and animals were identified. The EFSA GMO Panel is not aware of any new information that would change these conclusions. Updated bioinformatic studies[29] confirmed the absence of relevant similarities between these newly expressed proteins to known toxins. The potential for a functional interaction of the newly expressed desaturases and the CP4 EPSPS protein in the two-event stack soybean MON 87769 × MON 89788 has been assessed with regard to human and animal health. The two desaturase enzymes are intended to act in combination on plant fatty acid metabolism.

[29] Additional information: 10/07/2015.

The CP4 EPSPS enzyme catalyses a distinctly different biochemical reaction. No information was identified to suggest that the combination of the desaturases PjΔ6D and NcΔ15D with CP4 EPSPS would result in effects different from those observed in the single events. Since the individual proteins were considered safe for humans and animals, the same conclusion can be extended to their presence in the stacked soybean MON 87769 × MON 89788.

The EFSA GMO Panel concludes that there are no safety concerns for human and animal health related to the PjΔ6D, NcΔ15D and CP4 EPSPS proteins newly expressed in soybean MON 87769 × MON 89788.

4.3.2.2. Toxicological assessment of components other than newly expressed proteins

The compositional analysis of soybean MON 87769 × MON 89788 confirmed the expected altered fatty acid profile and a higher SDA level in seeds (see Table 5). All of these fatty acids occur naturally in the diet of humans and animals. The safety impact of the altered fatty acid profile is evaluated in Sections 4.3.4 and 4.3.5.

4.3.3. Animal studies with the food/feed derived from genetically modified plants

A 42-day feeding study with a total of 800 male and female (one-day-old Cobb 500) chickens for fattening was provided[30]. The birds were randomly allocated to eight dietary treatments with 100 chickens per treatment (five pens/treatment per gender, initially 12 birds per pen and reduced to 10 birds per pen at day seven). Birds were fed diets containing soybean MON 87769 × MON 89788 (verified by PCR in seeds), and compared with those fed diets containing the comparator (A3525) or any of the six non-GM commercial varieties (Anand, Ozark, NK S38-T8, H437, NC+2A86 and NK25-J5). The starter and grower/finisher diets consisted of 33 % and 30 % toasted meal, respectively. Other components were mainly maize and maize gluten meal (about 60 % and 63 % in the starter and grower/finisher diets, respectively). Before feed formulation, all soybean seeds were analysed for proximates, amino acids, minerals, vitamin E, antinutrients, mycotoxins and pesticides. The diets were isonitrogenous, isocaloric and balanced for limiting amino acids (confirmed by analysis). The starter diets (about 22 % crude protein (CP), 3 080 kcal metabolisable energy (ME) /kg) were given until day 21 and grower/finisher diets (about 20 % CP, 3 135 kcal ME/kg) were given from day 22 until the end. Feed (starter as crumbles and grower/finisher as pellets) and water were provided for *ad libitum* intake.

Chickens were observed twice daily for clinical signs; deaths were recorded and necropsy was performed on all birds found dead. Body weight per pen was measured at the start and the end of the trial. Feed intake was determined at day 21 and day 42 for each pen. At days 43 (males) and 44 (female) all surviving birds were taken for carcass evaluation (dressing percentage weight of thighs, breast, wings, drums, abdominal fat and whole liver). Data were analysed by a two-factor ANOVA (diet and sex) and pair-wise comparison was made by a Fischer's Least Significant Difference test. A mixed linear model was applied to compare soybean MON 87769 × MON 89788 with the mean of all non-GM varieties.

Overall mortality was low (< 3 %) with no significant difference between the groups. No significant treatment–sex interaction was detected for performance characteristics. Overall, no significant difference was seen in final body weight (about 2.6 kg), feed intake (about 3.9 kg), or feed to gain ratio (about 1.54) between soybean MON 87769 × MON 89788 and the comparator, or the comparator and the non-GM variety. No significant differences were observed in carcass characteristics.

No evidence of unintended effects introduced by the genetic modification was detected in the tested chickens. The Panel concluded that toasted soybean meal derived from MON 87769 × MON 89788 is as nutritious as the comparator and non-GM commercial varieties.

[30] Dossier: Part I— CQR-08-034 (2009) & RAR-10-168 (2010).

4.3.4. Allergenicity

For an allergenicity assessment, a weight-of-evidence approach is followed, taking into account all of the information obtained on the newly expressed proteins, since no single piece of information or experimental method yields sufficient evidence to predict allergenicity (EFSA, 2006a, 2011a; Codex Alimentarius, 2009). In addition, if 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 (EFSA, 2011a). If newly expressed proteins with a potential adjuvant activity are expressed together, possible interactions that might increase adjuvanticity and impact the allergenicity of the GM crop are assessed.

4.3.4.1. Assessment of allergenicity of the newly expressed proteins

With regard to allergenicity, the EFSA GMO Panel has previously evaluated the safety of the CP4 EPSPS, PjΔ6D and NcΔ15D proteins and no concerns were identified in the context of the applications assessed (e.g. EFSA, 2008, 2014). No new information on allergenicity of the newly expressed proteins that might change the previous conclusions of the EFSA GMO Panel has become available. Based on current knowledge, and since none of the newly expressed proteins showed allergenicity, no reasons for concern regarding the presence of these newly expressed proteins, in this stacked soybean, that affect allergenicity were identified.

As regards adjuvanticity, no information is available on the structure or function of the newly expressed CP4 EPSPS, PjΔ6D and NcΔ15D proteins that would suggest an adjuvant effect of the individual proteins or their presence in soybean MON 87769 × MON 89788 that would result in or increase an eventual IgE response to a bystander protein.

4.3.4.2. Assessment of allergenicity of the whole GM plant

Soybean is considered to be a common allergenic food[31] (OECD, 2012). Therefore, any potential change in the endogenous allergenicity of the GM plant when compared with that of its comparator(s) should be assessed (EFSA, 2011a). Such assessments were performed for the single-event soybeans MON 87769 and MON 89788, and no reasons for concern were identified by the EFSA GMO Panel (EFSA, 2008; EFSA GMO Panel, 2014).

At the request of the EFSA GMO Panel, the applicant provided an assessment of the endogenous allergenicity, comparing protein extracts of soybean MON 87769 × MON 89788 and its comparator by gel electrophoresis followed by mass spectrometry[32]. The intensities of the bands corresponding to specific allergens were analysed. No relevant changes in the allergen content between the protein extracts of soybean MON 87769 × MON 89788 and its comparator were identified.

The EFSA GMO Panel considers that there is no evidence that the genetic modification might significantly change the overall allergenicity of soybean MON 87769 × MON 89788 when compared with that of its comparator.

4.3.5. Nutritional assessment of genetically modified food/feed

4.3.5.1. Human nutritional assessment

The main product for human consumption from soybean is the oil. The nutritional consequences of the modifications in the fatty acid profile were assessed in the context of the previous opinion on the single event MON 87769 (EFSA GMO Panel, 2014).

[31] 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.
[32] Additional information: 11/09/2014.

In the context of this application, the applicant provided a dietary exposure and nutritional assessment based on data derived from the single event MON 87769, but not on soybean MON 87769 × MON 89788. Therefore, the applicant was asked to provide a dietary exposure assessment based on the compositional analysis of the RBD oil from soybean MON 87769 × MON 89788, taking into account different exposure scenarios, covering low and high consumer groups. However, the applicant did not provide this data[33]. The EFSA GMO Panel therefore cannot complete the assessment on the possible impact of soybean MON 87769 × MON 89788 oil on human health and nutrition.

Other soybean products for human consumption are not expected to differ in their composition, except for their fatty acid content. The contribution of fatty acids from such products to overall human exposure would be small and is not expected to affect the conclusion on human health and nutrition.

4.3.5.2. Animal nutritional assessment

Defatted toasted soybean meal represents the most common soybean by-product used in animal feed formulations, with around 90 % of the defatted soybean meal entering the feed chain in the EU for poultry, pigs and cattle. Presently, only small amounts of full-fat soybeans (1 % of the total soybean feed) are directly fed to food-producing animals. The use of soybean oil in animal feed is limited and only small amounts (0.5–3 %) are added to mixed feed (especially for poultry and pigs) in order to avoid dust, to improve the quality/stability of pellets and to add energy to the diets[34].

Compositional data indicates that the defatted soybean meal from soybean MON 87769 × MON 89788 would be expected to deliver the same nutrition as its comparator and other non-GM commercial varieties. This was confirmed by the results of a feeding study in chickens for fattening (see Section 4.3.3).

4.3.6. Post-market monitoring of genetically modified food/feed

As a full assessment on the possible health and nutritional impact of the soybean MON 87769 × MON 89788 oil was not made, the EFSA GMO Panel is not in the position to comment on the post-market monitoring plan and labelling.

4.3.7. Conclusion

The safety assessment identified no concerns regarding the potential toxicity and allergenicity of the newly expressed PjΔ6D, NcΔ15D and CP4 EPSPS proteins, and found no evidence that the genetic modification might significantly change the overall allergenicity of soybean MON 87769 × MON 89788. The EFSA GMO Panel could not complete a full assessment on the possible impact of the soybean MON 87769 × MON 89788 oil on human health and nutrition. There are no concerns regarding the use of feeding stuffs derived from defatted toasted MON 87769 × MON 89788 soybean meal.

4.4. Environmental risk assessment and monitoring plan

4.4.1. Evaluation of relevant scientific data

Considering the scope of application EFSA-GMO-NL-2010-85, the environmental risk assessment (ERA) of soybean MON 87769 × MON 89788 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 seeds of soybean MON 87769 × MON 89788 during transportation and processing.

[33] Additional information: 03/06/2015.
[34] Personal communication from Deutscher Verband für Tiernahrung, 29/07/2011.

As the scope of the present application excludes cultivation, environmental concerns in the EU related to the use of glyphosate-based herbicides on the GM soybean do not apply.

4.4.2. Environmental risk assessment

4.4.2.1. Potential unintended effects on plant fitness due to the genetic modification[35]

Cultivated soybean (*Glycine max* (L.) Merr.) is a species in the subgenus *Soja* of the genus *Glycine*. The species originated from eastern Asia and is a highly domesticated crop (Lu, 2005). The major worldwide soybean producers are Argentina, Brazil, China, North Korea, South Korea and the USA. In the EU, soybean is mainly cultivated in Italy, Romania, France, Hungary, Austria, Slovakia and the Czech Republic (Dorokhov et al., 2004; Krumphuber, 2008). Cultivated soybean seeds rarely display any dormancy characteristics and grow as volunteers in the year after cultivation under only certain environmental conditions. If volunteers occur, they do not compete well with the succeeding crop, and can easily be controlled mechanically or chemically (OECD, 2000). In soybean fields, seeds usually do not survive during the winter owing to herbivory, rotting and germination, or owing to management practices prior to planting the subsequent crop (Owen, 2005). Also, survival of soybean plants outside cultivation areas is limited mainly by a combination of low competitiveness, absence of a dormancy phase, and susceptibility to plant pathogens and cold climatic conditions.

The expected changes in seed fatty acid composition in soybean MON 87769 × MON 89788 resulting from the newly inserted *Pj.D6D* gene (encoding the Δ6 desaturase protein from *Primula juliae*) and the *Nc.Fad3* gene (encoding the Δ15 desaturase protein from *Neurospora crassa*) are not known to provide a potential agronomic advantage. The CP4 *epsps* gene-encoded herbicide tolerance trait does provide a potential agronomic and selective advantage for this GM soybean plant if glyphosate-based herbicides are applied.

Considering the scope of application EFSA-GMO-NL-2010-85, special attention is paid to those agronomic and phenotypic characteristics (for further details see Section 4.2) which may be indicative of changes in the survival of soybean MON 87769 × MON 89788 grains which could be accidentally released into the environment, as well as in the establishment and fitness of GM soybean plants, such as early and final stand count, yield, seedling vigour and 100 seed weight. As described in Section 4.2, all of these agronomic and phenotypic characteristics, except plant height, of soybean MON 87769 × MON 89788 did not differ from those of its comparator. Soybean MON 87769 × MON 89788 not treated with glyphosate-based herbicides had a higher plant height than its comparator in the across-site analysis. The measured values for this characteristic were within the natural range established using a set of reference varieties. The observed difference in plant height is unlikely to be biologically relevant in terms of increased persistence and invasiveness potential.

Specific data on pollen viability and seed germination for soybean MON 87769 × MON 89788 were not provided by the applicant. The EFSA GMO Panel considered the data[36] provided on seed germination for the single soybean events MON 87769 and MON 89788, their comparators and non-GM reference varieties. No statistically significant difference was observed in seed germination of soybean MON 87769 and soybean MON 89788 compared with their conventional counterparts across all sites. In addition, the early stand count data on soybean MON 87769 × MON 89788 indicated that changes in seed germination are unlikely.

Because the general agronomic and phenotypic characteristics that might be indicative of changes in survival, establishment and fitness are unchanged in soybean MON 87769 × MON 89788, herbicide tolerance is not likely to provide a selective advantage outside cultivation. Even if glyphosate-based herbicides are applied to these plants, this will not change their ability to survive over seasons. Therefore, it is considered very unlikely that soybean MON 87769 × MON 89788 will differ from

[35] Dossier: Part II—Section E 3.1 and Appendix D.
[36] Section D.4 of EFSA-GMO-NL-2006-36 and Section D.4 of EFSA-GMO-UK-2009-76.

conventional soybean varieties in its ability to survive until subsequent seasons or to establish feral populations under European environmental conditions.

The EFSA GMO Panel is not aware of any scientific report of increased survival capacity, including overwintering, of existing GM soybeans varieties, (Dorokhov et al., 2004; Owen, 2005; Bagavathiannan and Van Acker, 2008; Lee et al., 2009).

Therefore, the EFSA GMO Panel is of the opinion that the likelihood of environmental effects of soybean MON 87769 × MON 89788 in Europe will not be different from that of conventional soybean varieties.

4.4.2.2. Potential for gene transfer[37]

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.

Plant-to-bacteria gene transfer

The potential for horizontal gene transfer of the single events was assessed in previous opinions (EFSA, 2008, 2014) and no concern for 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, for instance because of combinations of recombinogenic sequences, which would cause an increase in the likelihood for horizontal gene transfer or a selective advantage were not identified.

Bioinformatic analysis of the inserted DNA and flanking regions (Section 3) did not identify sufficient sequence identity with bacterial DNA (including the modified CP4 *epsps* gene, which has been codon-optimised for expression in plants) that would facilitate homologous recombination-mediated gene transfer between plants and bacteria.

Therefore, the EFSA GMO Panel concludes that horizontal gene transfer from soybean MON 87769 × MON 89788 to bacteria is highly unlikely, theoretically possible but does not raise a safety concern.

Plant-to-plant gene transfer

Considering the scope of this application and the biology of soybean, a possible pathway of gene dispersal is through seed from accidental seed spillage during transportation and/or processing, and pollen from feral GM soybean plants.

The genus *Glycine* is divided into two distinct subgenera: *Glycine* and *Soja*. Soybean is in the subgenus *Soja*. The subgenus *Glycine* contains 16 perennial wild species, while the cultivated soybean, *Glycine max*, and its wild and semi-wild annual relatives, *G. soja* and *G. gracilis*, are classified in the subgenus *Soja* (OECD, 2000). Owing to the low level of genomic similarity among species of the genus *Glycine*, *G. max* can cross with only other members of the *Glycine* subgenus *Soja* under natural conditions (Singh et al., 1987; Hymowitz et al., 1998; Lu, 2005). Hence, the three species of the subgenus *Soja* are capable of cross-pollination and the hybrid seed that is produced can germinate normally and produce plants with fertile pollen and seed (Abe et al., 1999; Nakayama and Yamaguchi, 2002). Since *G. soja* and *G. gracilis* are indigenous to China, Taiwan, Korea, Japan, the far-east region of Russia, Australia, the Philippines and the South Pacific, and since they have not been reported in other parts of the world where the cultivated soybean is grown (Dorokhov et al., 2004; Lu, 2005), the plant-to-plant gene transfer from soybean is restricted to cultivated areas and occasional soybean plants resulting from seed spillage in the EU.

[37] Technical dossier/Part E/Section 3.2.

Soybean is an annual, almost completely self-pollinating crop with a percentage of cross-pollination usually lower than 1 % (OECD, 2000; Ray et al., 2003; Lu, 2005; Yoshimura et al., 2006; Abud et al., 2007). Soybean pollen dispersal is limited because the anthers mature in the bud and directly pollinate the stigma of the same flower (OECD, 2000).

However, cross-pollination rates as high as 6.3 % have been reported for closely spaced plants (Ray et al., 2003), suggesting the potential for some within-crop gene flow in soybean. These results indicate that natural cross-pollination rates can fluctuate significantly among different soybean varieties under particular environmental conditions, such as favourable climate for pollination and an abundance of pollinators (Gumisiriza and Rubaihayo, 1978; Kikuchi et al., 1993; Ahrent and Caviness, 1994; Ray et al., 2003; Lu, 2005).

For plant-to-plant gene transfer to occur, imported soybean MON 87769 × MON 89788 grains need to be processed outside the importing ports, transported into regions of soybean production in Europe, spilled during transportation, germinate and develop into plants in the very close vicinity of soybean fields, and there needs to be an overlap of flowering periods and environmental conditions favouring cross-pollination. It must be noted that most soybean MON 87769 × MON 89788 grains are processed in the countries of production or in ports of importation. The overall likelihood of cross-pollination between feral GM soybean plants and cultivated soybean is therefore extremely low.

In conclusion, as soybean MON 87769 × MON 89788 has no altered survival, multiplication or dissemination characteristics (see Section 4.4.2.1), the EFSA GMO Panel is of the opinion that the likelihood of environmental effects as a consequence of the spread of genes from this GM soybean in Europe will not differ from that of conventional soybean varieties.

4.4.2.3. Interactions of the GM plant with target organisms[38]

Considering the scope of application EFSA-GMO-NL-2010-85 and the absence of target organisms, potential interactions of the GM plant with target organisms were not considered a relevant issue by the EFSA GMO Panel.

4.4.2.4. Interactions of the GM plant with non-target organisms[39]

Considering the scope of application EFSA-GMO-NL-2010-85 and the low level of exposure to the environment, potential interactions of the GM plant with non-target organisms were not considered a relevant issue by the EFSA GMO Panel.

4.4.2.5. Interactions with the abiotic environment and biogeochemical cycles[40]

Considering the scope of application EFSA-GMO-NL-2010-85 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 EFSA GMO Panel.

4.4.3. Post-market environmental monitoring[41]

The objectives of a post-market environmental monitoring (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 genetically modified organism (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 EFSA GMO Panel gives its opinion on the scientific content of

[38] Technical dossier/Part D/Section 9.4.
[39] Technical dossier/Part D/Section 9.5.
[40] Technical dossier/Part D/Section 9.8.
[41] Technical dossier/Part D/Section 11.

the PMEM plan provided by the applicant (EFSA, 2006, 2011b). The potential exposure of the environment to soybean MON 87769 × MON 89788 would be through faecal material from animals fed the GM soybean or through accidental release into the environment of GM soybean seeds during transportation and processing. The EFSA GMO Panel is aware that, owing to the physical characteristics of soybean seeds and the methods of transportation, accidental spillage cannot be excluded. Also, it is important that appropriate management systems are in place to restrict seeds of soybean MON 87769 × MON 89788 entering cultivation as this would require specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

The PMEM plan proposed by the applicant includes (1) the description of a monitoring approach involving operators (federations involved in soybean import and processing), reporting to the applicants, 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 (Lecoq et al., 2007; Windels et al., 2008); and (3) the use of networks of existing surveillance systems. The applicant proposes to submit a PMEM report on an annual basis and a final report at the end of the consent period.

The EFSA 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-2010-85. As no potential adverse environmental effects were identified, case-specific monitoring was not considered necessary. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

4.5. Conclusion

No safety concerns with regard to the environment from the import and processing of soybean MON 87769 × MON 89788 were identified. There are no indications of an increased likelihood of the establishment and spread of feral soybean MON 87769 × MON 89788 plants in the case of accidental release into the environment of viable GM soybean seeds. The unlikely, but theoretically possible, transfer of recombinant genes from soybean MON 87769 × MON 89788 to bacteria does not give rise to a safety concern for these bacteria owing to the lack of a selective advantage. Potential interactions of soybean MON 87769 × MON 89788 with the biotic and abiotic environment were not considered a relevant issue by the EFSA GMO Panel. The PMEM plan provided by the applicant and the reporting intervals are in line with the scope of application EFSA-GMO-NL-2010-85.

CONCLUSIONS AND RECOMMENDATIONS

No new data on the single soybean events MON 87769 and MON 89788 that would lead to a modification of the original conclusions on their safety were identified.

The combination of the single soybean events MON 87769 and MON 89788 in the two-event stack soybean MON 87769 × MON 89788 did not give rise to issues, related to molecular, agronomic, phenotypic or compositional characteristics, regarding food and feed safety. The EFSA GMO Panel considers that there is no reason to expect interactions that could impact on the food and feed safety.

The safety assessment identified no concerns regarding the potential toxicity and allergenicity of the newly expressed PjΔ6D, NcΔ15D and CP4 EPSPS proteins, and found no evidence that the genetic modification might significantly change the overall allergenicity of soybean MON 87769 × MON 89788. Because of the lack of data on dietary exposure, based on the compositional analysis of RBD oil from soybean MON 87769 × MON 89788, the EFSA GMO Panel could not complete an assessment on the possible impact of MON 87769 × MON 89788 soybean oil on human health and nutrition. Therefore, the EFSA GMO Panel is not in the position to conclude on the food safety of soybean MON 87769 × MON 89788. There are no concerns regarding the use of feeding stuffs derived from defatted toasted MON 87769 × MON 89788 soybean meal.

No safety concerns with regard to the environment from the import and processing of soybean MON 87769 × MON 89788 were identified. There are no indications of an increased likelihood of

establishment and spread of feral soybean MON 87769 × MON 89788 plants in the case of accidental release into the environment of viable GM soybean seeds. The unlikely, but theoretically possible, transfer of recombinant genes from soybean MON 87769 × MON 89788 to bacteria does not give rise to a safety concern for these bacteria owing to the lack of a selective advantage. Potential interactions of soybean MON 87769 × MON 89788 with the biotic and abiotic environment were not considered a relevant issue by the EFSA GMO Panel. The PMEM plan provided by the applicant and the reporting intervals are in line with the scope of application EFSA-GMO-NL-2010-85.

In conclusion, the EFSA GMO Panel could not complete the food and feed safety assessment of soybean MON 87769 × MON 89788 because of the lack of an appropriate nutritional assessment. The EFSA GMO Panel concludes that soybean MON 87769 × MON 89788 is unlikely to have any adverse effect on the environment in the context of the scope of application EFSA-GMO-NL-2010-85.

As a full assessment on possible health and nutritional impact of soybean MON 87769 × MON 89788 oil was not made, the EFSA GMO Panel is not in the position to comment on the post-market monitoring plan and labelling provided by the applicant, in accordance with Articles 13(2)(a) and 25(2)(c) of Regulation (EC) No 1829/2003.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from Competent Authority of the Netherlands received on 30 July 2010 concerning a request for authorisation for the placing on the market of MON 87769 × MON 89788 soybean (application EFSA-GMO-NL-2010-85) submitted in accordance with Regulation (EC) No 1829/2003 by Monsanto Europe S.A./N.V.
2. Acknowledgement letter dated 9 September 2010 from EFSA to the Competent Authority of the Netherlands.
3. Letter from EFSA to applicant dated 9 September 2010 requesting additional information under completeness check.
4. Letter from applicant to EFSA received on 5 November 2010 providing additional information under completeness check.
5. Letter from EFSA to applicant dated 26 November 2010 delivering the "Statement of Validity" of application EFSA-GMO-NL-2010-85 (soybean MON 87769 × MON 89788) submitted by Monsanto Europe S.A./N.V under Regulation (EC) No 1829/2003.
6. Letter from EFSA to applicant dated 26 November 2010 stopping the clock because of single event.
7. Letter from applicant to EFSA received on 14 October 2013 spontaneously providing additional information.
8. Letter from EFSA to applicant dated 20 May 2014 re-starting the clock because of single event.
9. Letter from EFSA to applicant dated 14 July 2014 requesting additional information and stopping the clock.
10. Letter from EFSA to applicant dated 25 July 2014 requesting additional information and maintaining the clock stopped.
11. Letter from applicant to EFSA received on 12 September 2014 providing additional information.
12. Letter from applicant to EFSA received on 15 September 2014 providing additional information.

13. Letter from EFSA to applicant dated 10 November 2014 requesting additional information and maintaining the clock stopped.

14. Letter from applicant to EFSA received on 28 January 2015 providing additional information

15. Letter from EFSA to applicant dated 2 March 2015 re-starting the clock.

16. Letter from EFSA to applicant dated 30 March 2015 requesting additional information and stopping the clock.

17. Letter from applicant to EFSA received on 1 June 2015 providing additional information.

18. Letter from applicant to EFSA received on 10 July 2015 providing additional information.

19. Letter from EFSA to applicant dated 14 September 2015 re-starting the clock.

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