SCIENTIFIC OPINION

Scientific Opinion on application (EFSA-GMO-UK-2009-76) for the placing on the market of soybean MON 87769 genetically modified to contain stearidonic acid, 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]

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ABSTRACT

Soybean MON 87769 was developed using *Agrobacterium tumefaciens* transformation and was intended to modify the lipid profile of the extracted oil. Soybean MON 87769 contains a single insert consisting of the *Pj.D6D* gene encoding the Δ6 desaturase protein from *Primula juliae* and the *Nc.Fad3* gene encoding the Δ15 desaturase protein from *Neurospora crassa*, both involved in the desaturation of endogenous fatty acids into stearidonic acid. The molecular characterisation of soybean MON 87769 does not raise safety issues. Soybean MON 87769 differs from the conventional counterpart in its fatty acid profile. The safety assessment of the newly expressed desaturases identified no concerns regarding potential toxicity and allergenicity. Nutritional assessment of soybean MON 87769 and derived food products did not identify concerns about human health and nutrition. Consumption of MON 87769 soybean oil replacing other oils in food is not expected to result in adverse effects from increased SDA intake as shown in different exposure scenarios. There are no indications of an increased likelihood of establishment and spread of feral soybean plants. Considering the scope of the application, potential interactions of soybean MON 87769 with the biotic and abiotic environment were not considered a relevant issue. Environmental risks associated with a theoretically possible horizontal gene transfer from soybean MON 87769 to bacteria have not been identified. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of soybean MON 87769. Since the use of oil derived from the soybean MON 87769 will result in a higher intake of SDA, a post-market monitoring plan is recommended to confirm the exposure assessment using realistic consumption data for the European population.

KEY WORDS

GMO, soybean (*Glycine max* (L). Merr.), MON 87769, stearidonic acid, Regulation (EC) No 1829/2003, gamma-linolenic acid, alpha-linolenic acid

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[1] On request from the Competent Authority of the UK on an application (EFSA-GMO-UK-2009-76) submitted by Monsanto, Question No EFSA-Q-2009-00836, adopted on 9 April 2014.

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SUMMARY

Following the submission of an application (EFSA-GMO-UK-2009-76) under Regulation (EC) No 1829/2003 from Monsanto, the Panel on Genetically Modified Organisms (GMO) was asked to deliver a scientific opinion on soybean MON 87769 (unique identifier MON-87769-7), genetically modified (GM), to contain stearidonic acid, for food and feed uses, import and processing.

In delivering its scientific opinion, the EFSA GMO Panel considered the application EFSA-GMO-UK-2009-76, additional information supplied by the applicant, scientific comments submitted by Member States and relevant scientific publications. The scope of application EFSA-GMO-UK-2009-76 is for food and feed uses, import and processing of soybean MON 87769 and all derived products, but excludes cultivation in the European Union.

The EFSA GMO Panel assessed soybean MON 87769 with reference to the intended uses and appropriate principles described in the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of GM plants and derived food and feed (EFSA, 2006). The scientific assessment included molecular characterisation of the inserted DNA and the newly expressed proteins. A comparative analysis of agronomic traits and composition was undertaken, and the safety of the new protein and the whole food/feed were evaluated with respect to potential toxicity, allergenicity and nutritional quality. An assessment of environmental impacts and the post-market environmental monitoring plan were also undertaken.

Meristematic tissue excised from the embryos of germinated seeds of conventional soybean A3525 was transformed using *Agrobacterium tumefaciens* and expresses the *Primula juliae* Δ6 desaturase gene and the *Nc.Fad3* gene, which provides the expression of the *Neurospora crassa* Δ15 desaturase intended to modify the lipid profile of the extracted oil. The molecular characterisation data establish that the GM soybean MON 87769 contains a single insert consisting of the *Pj.D6D* and *Nc.Fad3* expression cassettes. No other parts of the plasmid used for transformation could be detected in soybean MON 87769. Bioinformatic analyses and genetic stability studies did not raise safety issues. The levels of the PjΔ6D and NcΔ15D proteins in soybean MON 87769 have been sufficiently characterised to inform the subsequent assessment.

A comparative analysis of soybean MON 87769 identified no phenotypic or agronomic differences with respect to its conventional counterpart (soybean A3525) and to non-GM soybean reference varieties. However, it confirmed that the composition of soybean MON 87769 differs from that of the conventional counterpart and non-GM soybean reference varieties. The newly expressed desaturases in soybean MON 87769 seeds resulted in an alteration of the fatty acid profile, leading to the appearance of four new fatty acids (stearidonic acid (SDA), γ-linolenic acid and two trans-fatty acids) and a reduction in linoleic acid (LA). The safety assessment identified no concerns regarding the potential toxicity and allergenicity of the newly introduced desaturase proteins. There are no indications that the genetic modification might change the overall allergenicity of soybean MON 87769 when compared with that of its conventional counterpart. The EFSA GMO Panel concludes that the estimated changes in fatty acid intake by consumers using oil from MON 87769 are unlikely to constitute a toxicological risk or to have negative nutritional consequences for humans.

Based on the results of studies in rats, it is concluded that feeding stuffs derived from defatted soybean MON 87769 are as safe and nutritious as those derived from other non-GM soybean varieties.

Based on different exposure scenarios, the EFSA GMO Panel concludes that the proposed use of MON 87769 soybean oil in foods is not expected to result in intakes of SDA with adverse effects and that the other changes in the dietary fatty acid pattern are unlikely to have negative nutritional consequences for humans. The EFSA GMO Panel notes that the quantitative dietary estimates described here would have to be revisited if the oil produced by soybean MON 87769 were to be extensively used in food products not considered in this assessment, for example as dietary supplements or to modify animal feed products.

The EFSA GMO Panel recommends a post-market monitoring plan to confirm the exposure assessment using consumption data for the European populations.

Considering the scope of application EFSA-GMO-UK-2009-76, there is no requirement for a scientific assessment of possible environmental effects associated with the cultivation of this GM soybean. There are no indications of an increased likelihood of establishment and spread of feral soybean MON 87769 plants in the case of accidental release into the environment of viable GM soybean seeds. Owing to the scope of application EFSA-GMO-UK-2009-76, 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. The theoretically possible transfer of the recombinant genes from soybean MON 87769 to environmental bacteria does not raise a concern owing to the lack of both an efficient transfer mechanism and an identified selective advantage. The scope of the post-market environmental monitoring (PMEM) plan provided by the applicant and the reporting intervals are in line with the intended uses of soybean MON 87769 and the guidance document of the EFSA GMO Panel on PMEM of GM plants (EFSA, 2011). In addition, the EFSA GMO Panel acknowledges the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure in cases of accidental release of viable seeds of soybean MON 87769. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

In conclusion, the EFSA GMO Panel considers that the information available for soybean MON 87769 addresses the scientific issues indicated by the Guidance document of the EFSA GMO Panel and the scientific comments raised by the Member States, and that soybean MON 87769 is as safe as its conventional counterpart and is unlikely to have adverse effects on human and animal health and the environment in the context of the scope of this application.

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BACKGROUND

On 20 October 2009, the European Food Safety Authority (EFSA) received from the Competent Authority of United Kingdom (UK) an application (Reference EFSA-GMO-UK-2009-76), for authorisation of genetically modified (GM) soybean MON 87769 submitted by Monsanto Europe S.A/NV within the framework of Regulation (EC) No 1829/2003[4] on genetically modified food and feed for food and feed uses, import and processing.

After receiving the application EFSA-GMO-UK-2009-76 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 26 January 2010, EFSA received additional information (requested on 27 November 2009). On 16 February 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 (until 16 May) to make their opinion known.

The scope defined by the applicant: "*includes all food and feed products containing, consisting or produced from* soybean MON 87769 *including products from inbreeds and hybrids obtained by conventional breeding of this soybean product. The application also covers the import and industrial processing of* soybean MON 87769 *for all potential uses as any other soybean.*"

The EFSA GMO Panel carried out an evaluation of the scientific risk assessment of the GM soybean MON 87769. On 3 may 2010, 5 August 2010, 13 October 2010, 20 July 2011, 7 February 2012, 9 January 2013 and 10 October 2013, the EFSA GMO Panel requested additional information. The applicant provided the requested information on 14 June 2010, 1 October 2010, 24 February 2011, 10 November 2011, 20 September 2012, 18 February 2013, 21 May 2013 and 3 January 2014, respectively.

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, the EFSA opinion shall include a report describing the assessment of the food and feed and stating the reasons for its opinion and the information on which its opinion is based. This document 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-2009-00444

[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.

TERMS OF REFERENCE

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

The EFSA GMO Panel was not requested to give a scientific opinion on information required under Annex II to the Cartagena Protocol. The EFSA GMO Panel did consider if there is a need for a specific labelling in accordance with Articles 13(2)(a) and 25(2)(c) of Regulation (EC) No 1829/2003. However, it did not consider proposals for methods of detection (including sampling and the identification of the specific transformation event in the food/feed and/or food/feed produced from it), which are matters related to risk management.

ASSESSMENT

1. Introduction

The scope of application EFSA-GMO-UK-2009-76 is for food and feed uses, import and processing of all derived products of soybean MON 87769 such as the production of refined oil and lecithin as food or food ingredients and the use of protein rich meal in animal feed.

Soybean MON 87769 (unique identifier MON-87769-7) was assessed with respect to its scope, taking account of the requirements described in the applicable guidance documents (EFSA, 2006). The risk assessment presented here is based on the information provided in the application submitted in the European Union (EU), scientific comments raised by the Member States and relevant scientific publications.

The genetic modification introduced in soybean MON 87769 results in the expression in the seeds of two novel desaturase enzymes intended to modify the lipid profile of the extracted oil. The first, a Δ15 desaturase, is active in the conversion of linoleic acid [(C18:2 (n-6)] (LA) to α-linolenic acid [C18:3 (n-3)] (ALA). The second enzyme, a Δ6 desaturase, promotes the conversion of ALA to octadecatetraenoic acid [C18:4 (n-3)], also known as stearidonic acid (SDA), which accumulates in the seed. The same enzyme also catalyses the conversion of LA to γ-linolenic acid [C18:3 (n-6)] (GLA), a precursor of arachidonic acid and the eicosanoids. SDA is a normal intermediate in the formation of the long chain omega-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid [(C20:5 (n-3)] (EPA) and docosahexaenoic acid [(C22:6 (n-3)] (DHA). However, in humans, the conversion of ALA to SDA is slow. Direct consumption of SDA avoids this step in the biosynthesis and can result in a more efficient synthesis of the higher chain-length PUFAs.

Three major processed fractions are produced from whole soybean seeds: oil, protein rich meal and lecithin. The oil derived from soybean MON 87769 is intended to be identity preserved to maintain its value and assure its appropriate use in food applications. According to the applicant, it is foreseen to be added to foods as an ingredient that provides a precursor for EPA and DHA, in most cases replacing a portion of other oils in the diet. The SDA soybean oil is intended to be used only by the food industry and, according to the applicant, will not be available as home-use oil.[7] The high content of PUFAs makes it unsuitable for high temperature operations such as frying.[8]

According to the applicant, the extracted protein rich meal will be used exclusively for animal feed. The applicant considers that, given the nature of the oil and separation from seeds of other soybean varieties needed to preserve identity, it is unlikely that whole soybean (full fat) or refined oil from MON 87769 would be used in animal diets. Nevertheless, the scope of the present application implies that soybean MON 87769 may be treated as any other soybean and this possibility is assessed below.

2. Issues raised by Member States

The comments raised by the Member States are addressed in Annex G of the EFSA overall opinion and were taken into consideration during the evaluation of the risk assessment.[9]

[7] Technical dossier p. 257.
[8] Technical dossier p. 258.
[9] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2009-00836

3. Molecular characterisation

3.1. Evaluation of relevant scientific data

3.1.1. Transformation process and vector constructs

Meristematic tissue excised from the embryos of germinated seeds of conventional soybean A3525 was transformed with the binary plasmid PV-GMPQ1972 using *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*) strain ABI. The plasmid PV-GMPQ1972 contained two T-DNAs. T-DNA I contained the *Pj.D6D* gene expression cassette providing the expression of *Primula juliae* Δ6 desaturase and the *Nc.Fad3* gene expression cassette, which provides the expression of the *Neurospora crassa* Δ15 desaturase. T-DNA II contained the CP4 *epsps* cassette conferring tolerance to glyphosate, which served as a selectable marker for transformation.[10] The two-T-DNA system enabled the cassettes encoding the traits of interest and the selectable marker to be inserted at two independent genetic loci within the genome of the soybean. After self-pollination of the transformed R0 plant, an R1 plant (designated as MON 87769) that contained a single T-DNA I, but not T-DNA II, was selected for further development.

The two T-DNAs present in plasmid PV-GMPQ1972 consisted of the following elements between their respective right and left border regions:

- T-DNA I (*Pj.D6D* and *Nc.Fad3* expression cassettes): seed-specific promoter and leader sequence (P-7Sα′) of soybean *Sphas1* gene (*Sphas1* gene encodes β-conglycinin, a 7Sα′ seed storage protein) to direct the transcription in the seed; coding sequence for the fatty acid Δ6 desaturase from *P. juliae* (primrose) (*Pj.D6D*); 3′ non-translated region of the *tml* gene from the *A. tumefaciens* octopine-type Ti plasmid that directs polyadenylation of the mRNA (T-*tml*); promoter and leader sequence (P-7Sα) of the soybean *Sphas2* gene (*Sphas2* gene encodes the α-subunit of β-conglycinin) to direct the transcription in the seed; coding sequence for the fatty acid Δ15 desaturase from *N. crassa* (*Nc.Fad3*); 3′ non-translated region of the *Pisum sativum* (garden pea) *rbcS2* gene that directs polyadenylation of the mRNA (*rbcS2* gene encodes Rubisco small subunit) (T-*E9*). The *Nc.Fad3* protein of *N. crassa* contained a single amino acid change (from threonine to alanine at the first amino acid after the start codon).

- T-DNA II (CP4 *epsps* expression cassette): FMV promoter (P-*FMV*) from *Figwort mosaic virus* 35S RNA gene, which drives transcription in most plant cells; 5′ non-translated leader sequence from the *Arabidopsis shkG* gene (*shkG* gene encodes EPSPS) to enhance expression (L-*ShkG*); sequence encoding the transit peptide region of *A. thaliana* EPSPS to direct the CP4 EPSPS protein to the chloroplast (TS-*CPT2*); modified coding sequence of the *aroA* gene from *Agrobacterium* sp. strain CP4, encoding the EPSPS protein, to confer tolerance to glyphosate during the selection of transformants (CS-CP4 *epsps*); 3′ non-translated region of the *P. sativum rbcS2* gene that directs polyadenylation of the mRNA (T-*E9*).

Additional functional elements in the plasmid vector outside the T-DNAs, and thus not expected to be transferred to the soybean genome, were: *oriV* origin of replication to maintain the plasmid in *Agrobacterium*; *ori-pBR322* origin of replication to maintain the plasmid in *Escherichia coli*; *rop* repressor of primer (ROP) protein to maintain plasmid copy number in *E. coli*; *aad*A bacterial selectable marker (promoter and coding regions) to confer spectinomycin/streptomycin resistance.[11]

3.1.2. Transgene constructs in the GM plant

The DNA sequences inserted in the MON 87769 event were characterised by Southern analysis and by polymerase chain reaction (PCR) amplification of both the insert and the flanking regions.[12]

[10] Dossier: Part I—Section C1, C2.
[11] Dossier: Part I—Section C3.
[12] Dossier: Part I—Section D2.

Southern analyses indicated that soybean MON 87769 contains a single insert with one copy of the intact *Pj.D6D* and *Nc.Fad3* expression cassettes. The insert and copy number were confirmed by multiple restriction enzyme/probe combinations covering the T-DNA region and flanking regions. No signal was observed with the overlapping probes corresponding to the PV-GMPQ1972 vector backbone and T-DNA II (except for the border sequences identical between the two T-DNAs). Some probes detected endogenous soybean sequences, as parts of the T-DNA I and T-DNA II cassettes were of soybean origin.

The nucleotide sequence of the entire insert, as well as approximately 1 kb of both 5′ and 3′ flanking regions (933 and 831 bp, respectively), were determined from soybean MON 87769. The sequence of the insert confirmed the conclusions drawn from the Southern analyses. The insert is identical to the T-DNA I of PV-GMPQ1972, except for the deletion of 313 bp of the right border and the deletion of 168 bp of the left border region. The possible interruption of known endogenous soybean genes by the insertion of event MON 87769 was evaluated by bioinformatic analyses of the pre-insertion locus and of the genomic sequences flanking the insert. Comparison of the sequences of the flanking regions in MON 87769 to those in the parental soybean A3525 indicated that in MON 87769 a 9 bp DNA segment of endogenous DNA has been deleted and two (17 and 8 bp) filler DNAs were introduced immediately 5′ and 3′ to the insertion site, respectively. BLASTN searches were performed against the GenBank EST (Expressed Sequence Tag) and non-redundant nucleotide database and BLASTX search against the GenBank non-redundant amino acid database. These bioinformatic analyses did not reveal the interruption of any known endogenous gene in the MON 88701 flanking regions.[13]

The results of segregation (see Section 3.1.4) and bioinformatic analyses established that the insert is located in the nuclear genome.[14]

In order to assess whether the open reading frames (ORFs) present within the insert and spanning the junction sites raise any safety issue, their putative translation products were compared with databases for similarities to known allergens and toxins using suitable algorithms. No significant similarities were found.[15]

3.1.3. Information on the expression of the insert

The levels of the integral membrane proteins PjΔ6D and NcΔ15D were estimated by semi-quantitative Western analysis with peptide antibodies developed against the soluble portion of the corresponding proteins. Data were analysed from replicated field trials in the USA across five locations in 2006 (n = 14) and five locations in 2007 (n = 15). As expected, since the newly expressed genes are under the control of seed-specific promoters, none of these proteins was detected in the leaf or root. In the 2006 growing season, the mean levels of PjΔ6D were estimated to be 16 µg/g dry weight (dw) (SD = 9.5 µg/g) with a range of 3.6–28 µg/g dw in forage (aerial plant parts including immature seeds), 100 µg/g dw (SD = 63 µg/g) with a range of 19–210 µg/g dw in immature seed and 1.8 µg/g dw (SD = 0.95 µg/g) with a range of 0.5–3.2 µg/g dw in mature seed. The NcΔ15D levels were estimated to be 14 µg/g dw (SD = 6.8 µg/g) with a range of 4.6–30 µg/g dw in forage, 200 µg/g dw (SD = 89) with a range of 66–330 µg/g dw in immature seed and 10 µg/g dw (SD = 6.5) with a range of 4.8–25 µg/g dw in mature seed. Similar levels were observed in the samples from 2007.[16] The levels of both proteins were markedly higher in immature seeds than in mature seeds for the two seasons and the highest levels were observed in the 2006 growing season. The immature seed was used as a source of both proteins for the toxicological assessment (Section 5.1.2.1).

3.1.4. Inheritance and stability of inserted DNA

The integration of the insert in the nuclear genome was confirmed by Southern analysis and PCR. Stability of the inserted DNA was studied by Southern analysis from four consecutive generations, all

[13] Dossier: Part I—Section D2. Additional information: 21/5/2013.
[14] Dossier: Part I—Section D2, D5. Additional information: 21/5/2013.
[15] Additional information: 21/5/2013.
[16] Dossier: Part I—Section D3.

of them produced by self-pollination (R3 to R6). The insert was stable and followed the Mendelian inheritance pattern of a single locus.[17] Phenotypic stability was indicated by analysing the presence of the T-*tml* 3′ genetic element (by quantitative structure-specific endonuclease-based assay) over three generations produced by self-pollination after an initial backcross of MON 87769 (R4 generation) with a conventional soybean variety.

3.2. Conclusion

The molecular characterisation data provided by the applicant establish that soybean MON 87769 contains a single insertion consisting of two intact expression cassettes (*Pj.D6D* and *Nc.Fad3*). No other parts of the plasmid used for transformation are present in the transformed plant. Bioinformatic analysis of the 5′ and 3′ flanking regions did not reveal disruption of known endogenous genes or regulatory sequences, or creation of ORFs that would cause a safety issue. The stability of the inserted DNA was confirmed over several generations and a Mendelian inheritance pattern was demonstrated. The EFSA GMO Panel concludes that the molecular characterisation does not raise safety issues.

The levels of the PjΔ6D and NcΔ15D proteins in soybean MON 87769, have been sufficiently characterised to inform the subsequent assessment.

4. Comparative analysis

4.1. Evaluation of relevant scientific data

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

Data on agronomic and phenotypic characteristics of soybean MON 87769, its conventional counterpart and a set of non-GM commercial varieties were collected in field trials performed in the USA in 2006 and 2007.[18] These field trials also supplied seed and forage material for compositional analysis of the various soybean materials. The composition of soybean MON 87769 was compared with that of the conventional counterpart Asgrow variety A3525, which was the commercial soybean variety originally used to establish transformation event MON 87769.

In both years, the field trial was carried out at five geographical sites representative of the soybean cultivation areas of the USA.[19] At each site, soybean MON 87769, the conventional counterpart and three non-GM commercial varieties were planted following a randomised complete block design with three replicates. All the soybeans at each field trial site were grown under normal agronomic management for that geographical region. In total, 10 different commercial non-GM soybean varieties were included in 2006 and 15 in 2007. These were cultivated to provide data on the natural variation in agronomic and phenotypic characteristics and composition amongst commercial soybean varieties. However, when an event-specific PCR analysis was made, one of the replicates in one of the reference lines from one of the field trials in 2007 was identified as contaminated with MON 87769 and excluded from the analysis. The reference material[20] was used to estimate a range of baseline values that are common to commercial soybean varieties for each parameter studied. On request from the EFSA GMO Panel, the applicant provided ranges of baseline values based only on non-GM soybean reference varieties.[21] All materials were grown at normal agronomic conditions for the specific geographical region. One of the field trial sites in 2006 was excluded from the analysis because two of the three control plots had poor soybean stands owing to a malfunction of the planting equipment.

[17] Dossier: Part I—Section D5.
[18] Technical Dossier/Section D7.1 i) and ii).
[19] The field trials in 2006 were performed at two sites in Iowa, and one site each in Illinois, Michigan and Ohio, and in 2007 at one site each in Iowa, Michigan, Nebraska, Pennsylvania and Wisconsin.
[20] In 2006 the reference lines were: A3244, ST3600, Stewart SB3454, **DKB34-51**, ST3608, **Pioneer 93M50**, Pioneer 93B82, Lewis 372, **AG3505**, CST3461 (STS), ST3300, CST37002, ST3870, A2869, ST2788, Lewis 392, A2804, and A2553. In 2007 they were: **DKB34-51**, Hoegemeyer 333, CST3461 (STS), ST3600, **AG3505**, ST3300, Stewart SB3454, CST37002, **Pioneer 93M50**, Midland 363, A3244 and ST3608 (reference lines in bold are GM soybeans).
[21] Additional information: 30/9/2010.

4.1.2. Agronomic traits and GM phenotype

The phenotypic and agronomic characteristics evaluated were early stand count, seedling vigour, plant growth stages, days to 50 % flowering, flower colour, plant pubescence, plant height, lodging, pod shattering, final stand count, seed moisture, 100-seed weight, test weight (g/250 ml) and yield. During each year, soybean MON 87769 was compared with soybean A3525 within each site and across sites. In the phenotypic comparison none of the parameters differed between soybean MON 87769 and the conventional counterpart in the statistical analysis across sites. In the individual site analysis, a total of 23 statistically significant differences were detected out of 204 comparisons. However, the mean values observed for soybean MON 87769 fell within the minimum and maximum mean values estimated for the reference lines. Therefore, the GMO Panel did not consider that these differences would require further assessment in the context of the scope of this application. No developmental differences (flower colour, plant pubescence and plant growth stage data) or altered pollen parameters (pollen diameter, morphology and viability) were observed between soybean MON 87769 and its conventional counterpart.

4.1.3. Compositional analysis

Soybean seeds were harvested from the field trials in the USA in 2006 and 2007, and analysed for proximates (protein, fat, ash and moisture), fibre fractions (acid detergent fibre (ADF) and neutral detergent fibre (NDF)), amino acids, fatty acids, vitamin E, anti-nutrients (i.e. phytic acid, trypsin inhibitor, lectins, stachyose and raffinose) and other secondary metabolites (isoflavones). Forage was analysed for proximates and for ADF and NDF. The 75 parameters analysed (68 in seeds and 7 in forage) were those recommended by OECD (2001) with the addition of extra fatty acids. Values for 26 endpoints frequently were at levels below the limit of quantification. When this occurred in more than 50 % of the samples, the analyte was omitted from the analysis.[22]

Comparison of forage parameters showed no statistically significant differences across locations in either season.

Statistically significant differences between the seed fatty acid composition of soybean MON 87769 and its conventional counterpart were observed as expected owing to the genetic modification. However, the total fat content of the seeds did not differ between soybean MON 87769 and its conventional counterpart. As shown in Table 1, the fatty acid composition in both years was qualitatively similar, although small differences in the proportions of the various fatty acids among years and sites were observed. In both years the most notable changes were a reduction in linoleic acid from 52.4–56.0 % to 16.5–30.8 % and in oleic acid from 17.2–21.5 % to 12.7–19.8 % of total fatty acids. This reduction was accompanied by the appearance of the two metabolites SDA (16.8–33.9 %) and GLA (6.1–8.0 %). In addition, low amounts of two trans-fatty acids previously not found in measurable concentrations in soybean oil, 9c,12c,15t trans-ALA (18:3) at 0.15–0.48 % and 6c,9c,12c,15t trans-SDA (C18:4) at 0.06–0.26 %, were detected.

The statistical analysis also revealed increased protein and reduced carbohydrate content in seeds. In agreement with this observation, the level of 17 of the 18 amino acids analysed was significantly increased in 2006 and the level 7 of the 18 increased in the following year. Differences in the levels of all amino acids were always within the variation defined by the soybean reference varieties included in the field trials. 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 a consequence of the altered

[22] Components excluded from the compositional analysis owing to predominant observations below the limit of quantitation of the assay were: 8:0 caprylic acid, 10:0 capric acid, 12:0 lauric acid, 14:0 myristic acid, 14:1 myristoleic acid, 15:0 pentadecylic acid, 15:1 pentadecenoic acid, 16:1 palmitoleic acid, 17:0 margaric acid, 17:1 heptadecylenic acid, 18:1 total trans fatty acids, 18:2 6c,9c isolinoleic acid, 18:2 total trans fatty acids, 18:3 gamma linolenic acid, 18:3 other 18:3 trans fatty acids, 18:3 9c,12c,15t trans ALA, 18:4 stearidonic acid (SDA), 18:4 6c,9c,12c,15t trans SDA, 20:2 11c,14c eicosadienoic acid, 20:3 11c,14c,17c eicosatrienoic acid, 20:4 arachidonic acid, 20:5 eicosapentaenoic acid (EPA), 22:1 erucic acid, 22:5 docosapentaenoic acid (DPA), 22:6 docosahexaenoic acid (DHA) and 24:0 lignoceric acid.

protein content. The EFSA GMO Panel identified no biological relevance in the observed altered protein and carbohydrate content of the soybean seeds requiring further assessment.

Table 1: Fatty acid profile (% of total fatty acids; mean and range) in seeds and in refined bleached deodorised oil produced from soybean MON 87769 and its conventional counterpart (A3525)

Compound		Fatty acid composition (% of total fatty acids) in MON 87769 soybean seeds						
		2006 USA (five locations)		2007 USA (five locations)		2006 USA (two locations)		Reference values*
		MON 87769	*A3525*	*MON 87769*	*A3525*	*MON 87769 RBD processed oil*	*A3525 RBD processed oil*	
Myristic acid (C14:0)	Mean	n.d.	n.d.	n.d.	n.d.	**0.083**	**0.082**	n.g.
	Range	n.d.	n.d.	n.d.	n.d.	0.078–0.088	0.078–0.089	n.d.
Palmitic acid (C16:0)	Mean	**12.06**	**11.77**	**12.40**	**11.80**	**12.10**	**11.48**	**9.55–15.77**
	Range	11.53–12.54	11.14–12.08	12.13–12.77	11.63–12.11	11.98–12.23	11.42–11.61	9.88–12.33
Palmitoleic acid (C16:1)	Mean	n.d.	n.d.	n.d.	n.d.	**0.085**	**0.091**	n.g.
	Range	n.d.	n.d.	n.d.	n.d.	0.083–0.087	0.088–0.095	n.d.
Heptadecanoic acid (C17:0)	Mean	n.d.	n.d.	n.d.	n.d.	**0.10**	**0.096**	n.g.
	Range	n.d.	n.d.	n.d.	n.d.	0.090–0.11	0.088–0.11	n.d.
Stearic acid (C18:0)	Mean	**4.19**	**4.15**	**4.25**	**4.12**	**4.18**	**4.08**	**2.70–5.88**
	Range	3.73–4.53	3.85–4.44	4.05–4.52	3.96–4.33	4.13–4.20	4.04–4.12	3.61–4.93
Oleic acid (C18:1)	Mean	**15.18**	**19.19**	**17.98**	**20.37**	**16.02**	**19.25**	**14.3–32.2**
	Range	12.66–18.80	17.24–21.17	16.89–19.80	19.35–21.52	14.49–17.34	19.02–19.74	16.70–26.06
6c,9c Isolinoleic acid (C18:2)	Mean	n.d.	n.d.	n.d.	n.d.	**0.091**	**0.075**	n.g.
	Range	n.d.	n.d.	n.d.	n.d.	0.089–0.094	0.063–0.085	n.d.
Linoleic acid (C18:2)	Mean	**22.78**	**54.93**	**24.50**	**54.25**	**25.66**	**55.38**	**42.3–58.8**
	Range	16.46–30.81	54.05–56.04	22.15–27.58	52.44 –55.29	20.66–30.92	54.82–55.87	51.08–58.44
α-Linolenic acid (C18:3)	Mean	**11.18**	**9.20**	**10.42**	**8.68**	**10.61**	**8.31**	**3.00–12.52**
	Range	10.20–11.80	7.42–10.66	10.12–10.97	8.07–9.16	10.34–10.95	7.42–9.07	6.95–10.58
Trans-α-Linolenic acid (C18:3) 9c,12c,15t	Mean	**0.44**	n.d.	**0.21**	n.d.	**0.51**	**0.14**	n.g.
	Range	0.38–0.48	n.d.	0.15–0.25	n.d.	0.47- 0.54	0.10–0.16	n.d.
Other trans-linolenic acids (C18:3)**	Mean	n.d.	n.d.	n.d.	n.d.	**0.064**	**0.084**	n.g.
	Range	n.d.	n.d.	n.d.	n.d.	0.031- 0.078	0.069–0.098	n.d.
γ-Linolenic acid (C18:3)	Mean	**7.09**	n.d.	**6.94**	n.d.	**6.68**	n.d.	n.g.
	Range	6.07–8.03	n.d.	6.36–7.27	n.d.	6.19–7.19	n.d.	n.d.
Stearidonic acid (C18:4)	Mean	**26.13**	n.d.	**22.35**	n.d.	**22.62**	n.d.	n.g.
	Range	16.83–33.92	n.d.	19.53–24.46	n.d.	16.88–28.35	n.d.	n.d.
Trans-Stearidonic acid (C18:4) 6c,9c,12c,15t	Mean	**0.18**	n.d.	**0.15**	n.d.	**0.26**	n.d.	n.g.
	Range	0.058–0.26	n.d.	0.062–0.19	n.d.	0.17–0.39	n.d.	n.d.
Arachidic acid (C20:0)	Mean	**0.34**	**0.31**	**0.35**	**0.31**	**0.35**	**0.31**	**0.16–0.48**

Compound		Fatty acid composition (% of total fatty acids) in MON 87769 soybean seeds						
		2006 USA (five locations)		2007 USA (five locations)		2006 USA (two locations)		Reference values*
		MON 87769	*A3525*	*MON 87769*	*A3525*	*MON 87769 RBD processed oil*	*A3525 RBD processed oil*	
	Range	0.31–0.37	0.28–0.34	0.33–0.37	0.29–0.33	0.34–0.35	0.30–0.33	0.27–0.36
Eicosenoic acid (C20:1) 11c	Mean	**0.14**	**0.13**	**0.18**	**0.16**	**0.18**	**0.17**	n.g.
	Range	0.075–0.20	0.069–0.19	0.17–0.19	0.079–0.19	0.16–0.20	0.14–0.19	0.071–0.19
Behenic acid (C22:0)	Mean	**0.29**	**0.32**	**0.29**	**0.30**	**0.32**	**0.33**	**0.28–0.60**
	Range	0.26–0.31	0.28–0.37	0.27–0.30	0.28–0.32	0.27–0.35	0.29–0.36	0.29–0.41
Lignoceric acid (C24:0)	Mean	n.d.	n.d.	n.d.	n.d.	**0.093**	**0.12**	n.g.
	Range	n.d.	n.d.	n.d.	n.d.	0.076–0.11	0.10–0.14	n.d.

Statistically significant differences (p = 0.05) and newly appearing compounds are shown on a shaded background.
n.g. = not given; n.d. = not detectable; RBD = refined, bleached and deodorised.
*Bold in this column: data from ILSI (2008), whereas non-bold data refer to reference lines in field trials in the USA in 2006 and 2007.
**The other trans-(C18:3) linolenic acids were 9c,12t,15c-; 9t,12c,15t-; and 9t,12c,15-(C18:3) linolenic acid.

A higher vitamin E content in soybean MON 87769 was observed at only one of the five sites in 2007, but not in 2006. Of the anti-nutrients, the phytic acid level was increased in soybean MON 87769 in 2007. However, the levels were within the 99 % tolerance interval of the levels of the reference soybean varieties. A reduction in daidzein and genistein content (30–36 %) in 2006 and 2007 and in glycitein level (only in 2006) were also observed.[23] However, these reduced isoflavone levels were within the 99 % tolerance interval of the reference soybean varieties included in the field trials. There were several additional statistically significant differences identified in the per location statistical analysis, but the levels observed in soybean MON 87769 were within the range of values observed in the reference varieties and therefore did not raise concern.

4.2. Conclusion

A comparison of soybean MON 87769 with its conventional counterpart (soybean A3525) and non-GM soybean reference varieties identified no phenotypic or agronomic differences requiring further assessment. The newly expressed desaturases in soybean MON 87769 seeds resulted in an alteration of the fatty acid profile; this alteration is characterised by the appearance of four new fatty acids (SDA, GLA and two trans-fatty acids) coupled with the reduction in LA. The safety and nutritional impacts of the altered fatty acid levels are evaluated in Section 5.

The EFSA GMO Panel identified no biological relevance in the other observed differences which, therefore, do not require further assessment.

5. Food/feed safety assessment

5.1. Evaluation of relevant scientific data

5.1.1. Effect of processing

Soybeans were harvested from two of the five sites in the USA in 2006[24] in order to perform compositional analyses on processed fractions, including defatted and toasted meal; refined, bleached and deodorised oil; protein isolate; and crude lecithin, derived from MON 87769, A3525 and eight conventional reference soybean varieties. The soybean meal was analysed for proximates, fibre fractions, amino acids, fatty acids, phytic acid and trypsin inhibitors, the soybean oil for fatty acids and vitamin E, the protein isolate for amino acids, fatty acids and moisture and, finally, crude lecithin for fatty acids and phosphatides. In all cases where a fatty acid analyses was made, the compounds analysed were the extended battery of fatty acids defined in Section 4.1.2. In total, 129 analytes were determined in the compositional comparison of soybean products.

Comparing the defatted and toasted meal[25] produced from soybean MON 87769 with similar meals from the conventional counterpart showed changes in the fatty acid profile that mirrored the differences seen with the whole soybean (i.e. reduced LA and oleic acid and the appearance of SDA and GLA). Statistically significant changes in the concentration of six other constituents were also found. These were slight increases in the level of four amino acids and in ADF in soybean MON 87769 and a reduction in the calculated carbohydrate content. The fatty acid changes were expected owing to the genetic modification. The small increase in amino acid content and the reduced carbohydrate content of the meal mirrored the differences observed in the whole seed as would be expected. These changes have been previously considered (see Section 4.1.3.) and found to be of no biological relevance.

As expected, refined, bleached and deodorised oil from soybeans MON 87769 differs from that of the corresponding oil produced from A3523 soybean. When the compositional data on processed oils from both types of soybean were compared (Table 1), statistically increased levels of palmitic acid, stearic acid, trans-ALA and vitamin E were observed, whereas the level of lignoceric acid was reduced. The

[23] Technical Dossier/Section D7.
[24] Technical Dossier/Section D7.1 iii).
[25] Frequently containing around 1 % fat (not more than 3 %) compared with 20 % in full fat meal.

level of LA was extensively reduced (from 54.8–55.9 % in the conventional counterpart to 20.7–30.9 % of the fatty acids in soybean MON 87769). In addition to these changes, three of the new fatty acids identified in the whole seed were also seen in the refined oil from MON 87769 (SDA, GLA and trans-SDA, constituting 16.9–28.4 %, 6.2–7.2 % and 0.17–0.39 % of the total fatty acids respectively). Small quantities of trans-ALA were present in all types of refined, bleached and deodorised soybean oil, suggesting that small quantities of this trans-fatty acid may be produced during processing of the oil. Owing to the lack of commercially available standards for 9c,12t,15c, 9t,12c,15t and 9t,12c,15c C18:3 trans-fatty acids, these could not be individually quantified.[26] However, the sum of these "other C18:3 trans-fatty acids" was similar in processed oil of soybean MON 87769 (0.031–0.078 %), soybean A3525 (0.069–0.098 %) and reference soybeans (0.031–0.083 %). The total trans-fatty acid levels in commercial grade SDA soybean oil was reported to range from 0.5 % to 0.8 % of total fatty acids. Besides the reduced level of linoleic acid (C18:2), and the new fatty acids in oil produced from soybean MON 87769, changes in the level of the various fatty acids (and vitamin E) normally found in conventional soybean oil were small, and were within the 99 % tolerance interval of the level of the various fatty acids defined by oil produced from the conventional reference soybean varieties included in the study.

On request from the EFSA GMO Panel, the applicant supplied information on the oxidative stability of the SDA enriched oil obtained from soybeans MON 87769.[26] At room temperature (25 ºC in air) the oil maintains acceptable quality for at least 72 days, while under accelerated ageing conditions (55 ºC in air) it is substantially shorter; however, it kept an acceptable quality for at least four to five days. These data indicate that the transition times for SDA soybean oil at the accelerated ageing conditions are within the ranges observed for other omega-3 oils, such as stabilised fish and algal oils. Storage of the SDA soybean oil under nitrogen at room temperature maintained the quality of the oil for at least nine months (FDA, 2009).

A comparison of the composition of protein isolates from soybean MON 87769 and soybean A3525 revealed a slightly reduced level of leucine, which was within the 99 % tolerance interval of the conventional reference soybean varieties. No other significant differences were observed other than those related to the lipid content. Protein isolate is derived from defatted soy flour and therefore contains even lower levels of lipids, typically 3 % total fat. As expected, the pattern of individual lipids found reflected the starting material: LA in protein isolate from soybean MON 87769 was reduced, and trans-ALA and ALA increased. The fat phase of the protein isolate produced from soybean MON 87769 also contained SDA, GLA and trans-SDA.

Comparing the composition of crude lecithin produced from MON 87769 and soybeans A3525 harvested across sites revealed no difference in the concentration of the four phosphatides investigated. Whereas the level of arachidic acid (C20:0) was increased, the level of lignoceric acid (C24:0) was reduced, but the levels of these fatty acids where within the 99 % tolerance interval of the conventional reference soybean varieties. The level of linoleic acid (C18:2) was reduced from 57.3–58.7 % of total fatty acids in soybean A3525 to 22.1–34.3 % of total fatty acids in soybean MON 87769. The crude lecithin derived from soybean MON 87769 contained SDA, GLA and trans-SDA, which are usually not detected in lecithin from conventional soybeans.

In conclusion, the comparative compositional analyses of products derived from soybean MON 87769, including defatted and toasted meal; refined, bleached and deodorised oil; protein isolate; and crude lecithin, identified that, besides the expected changes in fatty acid composition, levels of other analysed constituents in soybean MON 87769 either were comparable with those in the conventional counterpart (soybean A3525) or, when significantly altered, were within the range of that particular constituent observed in products processed from the reference soybean varieties.

[26] Additional information: 23/2/2011.

5.1.2. Toxicology

This assessment concentrates on the newly expressed proteins *Primula juliae* Δ6 desaturase (PjΔ6D) and *Neurospora crassa* Δ15 desaturase (NcΔ15D) and on the four fatty acids stearidonic acid (C18:4; SDA), γ-linolenic acid (C18:3; GLA), 9c,12c,15t trans-ALA (C18:3) and 6c,9c,12c,15t trans-SDA (C18:4) produced in seeds of soybean MON 87769 normally not present at detectable levels in non-GM soybean seeds.

5.1.2.1. Proteins used for safety testing[27]

The newly expressed PjΔ6D and NcΔ15D proteins used for safety testing were extracted from immature soybean MON 87769 seeds by solubilisation from membranes and subsequent purification by chromatographic procedures.

The PjΔ6D protein in the extract had a concentration of 0.52 mg/ml, a purity of 47 % and an apparent molecular weight of 45.9 kDa as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The 15 N-terminal amino acids of the PjΔ6D protein were shown to correspond to the amino acids deduced to constitute the coding region of the PjΔ6D gene present in soybean MON 87769, except for the terminal methionine, which is absent from the purified enzyme. It is known that post-translational modification of proteins frequently removes N-terminal methionine residues (Bradshaw et al., 1998). Matrix-assisted laser desorption ionisation time-of-flight (MALDI-TOF) analysis confirmed the identity of the MON 87769-produced PjΔ6D. A glycosylation assay identified no glycosylated proteins with a size around 46 kDa.

The NcΔ15D protein in the extract had a concentration of 0.62 mg/ml, a purity of 74 % and an apparent molecular weight of 46.2 kDa as determined by SDS-PAGE. Also in this case the 15 N-terminal amino acids of the NcΔ15D protein corresponded to the amino acids deduced from the coding region of the MON 87769 soybean NcΔ15D gene, except that the terminal methionine was again absent in the purified enzyme. MALDI-TOF analysis confirmed the identity of the MON 87769-produced NcΔ15D. A glycosylation assay identified no glycosylated proteins with a size around 46 kDa.

The functional activities of both enzymes were investigated using a *Saccharomyces cerevisiae* PjΔ6 or NcΔ15 desaturase-expressing system. *In vitro* functional activity was also demonstrated for PjΔ6 desaturase, demonstrating ^{14}C-labelled stearidonic acid synthesis from ^{14}C-ALA-CoA incubated with crude homogenates of young, fresh MON 87769 seeds. For methodological reasons (lack of sensitivity of the method) it was not possible to demonstrate the functional activity of the NcΔ15 desaturase in soybeans MON 87769.

The two protein preparations were used in *in vitro* degradation studies, in heat denaturation studies and in acute toxicity studies in mice.

5.1.2.2. Assessment of newly expressed proteins[28,29]

The newly expressed proteins encoded by the desaturase genes in soybean MON 87769 naturally occur in the flowering plant *Primula juliae* and the ascomycete fungus *Neurospora crassa.* Only *Neurospora crassa* is used for food preparation in some regions of the world. Onchom is a soybean-based press cake with *N. crassa* frequently consumed in Indonesia (Matsuo, 1997). In Brazil, *N. crassa* is used to process cassava in preparing a fermented drink (Park et al., 1982), and in France *N. crassa* is used in cheese production (Perkins and Davis, 2000).

The PjΔ6D and NcΔ15D proteins expressed in soybean MON 87769 are homologous to desaturase proteins universally present in the human diet. Bioinformatics-supported searches in databases showed that the amino acid sequence of the PjΔ6D protein shares partial identity with other "front-end"

[27] Technical Dossier/Section 7.8.1a.
[28] Technical Dossier/Section 7.8.1b.
[29] Additional information: 23/12/2013.

desaturases naturally occurring in plants used for food production (e.g. *Camellia sinensis*: beverage tea, 66 % identity; peanut, 64 %; banana, 63 %; turnip, 56 %; maize, 55 %[30]), in the rainbow trout (27 %) and common carp (25 %). Similarly, the NcΔ15D shares partial amino acid identity with the "omega" desaturases in, for example, various *Brassica* vegetables, parsley, soybean, peanut, olive, wheat, potato, tomato and apple (showing 44–28 % identity).

(a) *In vitro* degradation by proteolytic enzymes[31]

The resistance to degradation by pepsin of the PjΔ6D protein isolated from soybean MON 87769 seeds was studied in solutions at pH ~ 1.2. The integrity of the test protein in samples taken at various time points was analysed by SDS-PAGE followed by protein staining or Western blot. No intact protein (ca. 46 kDa) was seen within 30 seconds of incubation. A fragment of around 10 kDa observed after half a minute of incubation was no longer seen after two minutes. Several shorter fragments were also observed after different incubation periods. These fragments were most likely to be from co-purified proteins as they were not identified by Western blot analysis or N-terminal sequencing. The origin of a 5 kDa fragment could not be established.

The resistance to degradation by pepsin of the NcΔ15D protein isolated from soybean MON 87769 seeds was studied following the same methods used for the PjΔ6D protein. No intact protein (ca. 46 KDa) was seen within 30 seconds of incubation using SDS-PAGE and colloidal blue gel staining. In this study, several shorter fragments were observed after different incubation periods. N-sequencing of the smaller fragments showed that a 4 kDa fragment matched the sequence of the NcΔ15D protein. Other smaller fragments were most likely co-purified proteins as they were not identified by Western blot analysis or N-terminal sequencing. Two larger fragments (i.e. ~ 17 kDa and ~ 12 kDa), which could be observed up to 5 and 10 minutes, respectively, were detected by Western blotting methods and were assumed to be degradation products of the NcΔ15D protein.

(b) Heat denaturation

The newly expressed proteins showed a significant loss of immunoreactivity (approximately 90 %) as determined by Western blot and were not significantly detected by SDS-PAGE after 15 minutes at 95 °C. In these conditions, the appearance of higher molecular weight immunodetectable species was noted at Western blot analysis.

(c) Acute toxicity testing[32]

The applicant provided single-dose oral toxicity studies in which the PjΔ6 desaturase and the NcΔ15 desaturase isolated from soybean MON 87769 were administered to CD-1 mice (10 males and 10 females per treatment group). No adverse effects were observed at a dose (purity-corrected) of 4.66 mg/kg body weight (bw) for the PjΔ6 desaturase and 37.3 mg/kg bw for the NcΔ15 desaturase.

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

(d) 28-day repeated dose oral toxicity studies

Although requested, the applicant was unable to provide 28-day repeated dose studies on the newly expressed proteins, owing to technical difficulties in obtaining purified proteins in an amount suitable for toxicological studies. PjΔ6 and NcΔ15D desaturases expressed in soybean MON 87769 are integral membrane proteins, spanning the membrane lipidic bilayer and strongly interacting with the lipidic aliphatic portion. Protocols for isolating these from the plant are not scalable and lead to inactivation of the proteins. Equivalence of the proteins obtained by an *Escherichia coli* system was

[30] Additional information: 23/12/2013.
[31] Technical Dossier/Section 7.9.1 iv).
[32] Technical Dossier/Section 7.8.1b.

not demonstrated owing to presence of tightly associated lipids; furthermore, the amount of the required detergents needed for its extraction might be toxic to the animal system in a 28-day study. The applicant also used other heterologous systems for the expression of PjΔ6 or NcΔ15 desaturases, including yeast and insect cells, but in each case the expression level was too low to generate sufficient material for repeated dose toxicological studies.[33]

(e) Bioinformatic studies

Bioinformatic analyses of the amino acid sequences of the PjΔ6D and NcΔ15D proteins expressed in soybean MON 87769 showed no relevant similarities with known toxic proteins.[34,35]

(f) Conclusion on the safety of the newly expressed proteins

The Panel requested 28-day toxicity studies on the newly expressed proteins to confirm their safety in the absence of a history of consumption of these specific proteins. However, according to the applicant, it was not possible to generate sufficient protein preparations of suitable quality. The Panel accepted the technical reasons why the studies could not be performed. In the absence of these studies, the Panel considered the data available and took a weight-of-evidence approach to reach its conclusions based on the following considerations:

- Bioinformatics did not reveal amino acid sequence homology of these proteins with known toxins.
- The PjΔ6D and NcΔ15D proteins are integral membrane fatty acid desaturases. The scientific literature does not indicate that known toxic proteins have such desaturase activity as a component of their biological activity.
- Humans and animals consume other desaturases daily with no reported adverse effects.
- The PjΔ6D and NcΔ15D proteins in the preparations tested were rapidly degraded by pepsin *in vitro*.

The Panel considers that this information reduces the uncertainty raised by the lack of 28-day repeated dose toxicity studies on these newly expressed proteins and concludes that there are no reasons to suppose that these specific desaturases would introduce safety concerns.

5.1.2.3. Assessment of new constituents other than proteins and/or changed levels of natural constituents

This section focuses on the toxicological assessment, based on published studies in humans and animals, of four fatty acids found in higher amounts in MON 87769 than in conventional soybean: SDA, GLA, 9c,12c,15t trans-ALA (18:3) and 6c,9c,12c,15t trans-SDA (see Section 4.1.2).

(a) 18:4 n-3 Stearidonic acid[36]

Human studies

In intervention studies on humans with various amounts of SDA ethyl esters and/or SDA-containing plant derived oils, and with SDA-enriched soybean oil for between 14 and 84 days and at doses ranging from 0.05 to 4.2 g SDA/day, no adverse effects were reported (weight, serum lipids, immune parameters, clinical symptoms) (Diboune et al., 1992; James et al., 2003; Miles et al., 2004a, 2006; Surette et al., 2004; Lemke et al., 2010; Kuhnt et al., 2014). Neither SDA nor its elongation product 20:4 n-3 (eicosatetraenoic acid) accumulated at detectable levels in plasma phospholipids and blood

[33] Additional information: 23/12/2013.
[34] Technical Dossier/Section 7.8.1 ii). Additional information: 17/5/2013.
[35] Additional information: 23/12/2013.
[36] Dossier: Part I—Section 7.8.3 and Annex I.

cells (erythrocytes, platelets and mononuclear cells) and in plasma cholesterol esters and triglycerides after three weeks of consumption of 0.75 or 1.5 g SDA/day (Diboune et al., 1992; James et al., 2003; Miles et al., 2004a).

Animal studies

For the safety assessment of SDA, the applicant referred to feeding studies in which rats were supplied up to 1.04 g SDA/kg bw/day in the form of soybean oil. No adverse effects were seen in any of these studies (see Section 5.1.3).

It should be noted that PUFA Δ-6 desaturation and elongation are more pronounced in rodents than in humans, and extrapolation of study results to humans should be done with caution (Whelan, 2009).

(b) 18:3 n-6 γ-linolenic acid[37]

Human studies

In several studies, human diets were supplemented with GLA at doses from 1 to 5 g/day for periods of one to six months, leading to increased accumulation of GLA and dihomo-γ-linolenic acid (DGLA) in plasma and blood cell lipids (Barre and Holub, 1992; Leventhal et al., 1993; Guivernau et al., 1994; Zurier et al., 1996; Kenny et al., 2000; Middleton et al., 2002; Fewtrell et al., 2004; Miles et al., 2004a; Stoney et al., 2004; Kalantar-Zadeh et al., 2005), in general without significant increases in arachidonic acid. These doses have been well tolerated and no serious adverse effects were reported.

Animal studies

The applicant referred to 13 scientific reports on repeated dose toxicity studies38 in experimental animals (mainly in male rats but also in mice, guinea pigs and dogs) supplied GLA in the diet (usually as a constituent of plant oils), aiming to study beneficial as well as adverse effects. These studies, which supplied doses up to 6 000 mg GLA/kg bw for, depending on the study, 3 to 52 weeks, indicated no adverse effects of the dietary exposure to oils containing GLA.

(c) Trans-fatty acids[39]

It is assumed that trans-SDA is mainly formed by trans-isomerisation of unsaturated fatty acids during the processing of the oil (see Section 5.1.1). No specific studies have looked at the effects of consuming trans-SDA.[40]

Like ALA, trans-ALA isomers are oxidised (Bretillon et al., 2001), incorporated in plasma lipids and converted to long chain PUFAs (Sébédio et al., 2000). The daily intake of trans-ALA in French subjects is estimated to be 0.2–0.4 g (Sébédio et al., 2000). In the TRANSLine study, male healthy volunteers were after six weeks on a trans-free diet given a diet providing 0.6 percentage of energy (E%) as trans-ALA (Armstrong et al., 2000). The altered diet did not affect platelet aggregation, platelet thromboxane production, fibrinogen levels, factor VII, activated factor VIIa or plasminogen activator inhibitor activity in the studied males. However, there was an increase in the ratio of low-density lipoprotein (LDL) cholesterol to high-density lipoprotein (HDL) cholesterol in the plasma and in the total cholesterol/HDL cholesterol ratio (Vermunt et al., 2001). On this basis it was concluded that adverse effects of trans-fatty acids with regard to human lipid metabolism are dose dependent and have been observed to occur at intakes above 0.6 E%.

[37] Dossier: Part I—Section 7.8.3 and Annex I.
[38] Dossier: Part I—Appendix A.
[39] Dossier: Part I—Section 7.8.3 and Annex I. Additional information: 23/2/2011.
[40] Additional information: 23/2/2011.

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

5.1.3.1. Sub-chronic toxicity study with defatted soybean meal

In a 90-day feeding trial Sprague–Dawley Crl:CD rats (20 individually housed animals per sex and group) were fed *ad libitum* diets containing 5 % or 15 % processed (defatted) meal of soybean MON 87769 or the conventional counterpart (A3525).[41] The diet containing 5 % processed MON 87769 soybean meal was complemented with 10 % soybean meal of A3525 to provide a consistent 15 % content of soybean meal. The diets were nutritionally balanced and analysed for their composition. Statistical analysis (one-way analysis of variance (ANOVA) followed by Dunnett's test when $p < 0.05$) was performed on body weight, body weight gain, food consumption, clinical pathology and organ weight data. Microscopic findings were compared using Fisher's exact test.

One of the female rats in the low dose group (5 % MON 87769) was found dead on day 60 of the study, with no cause for the death identified; in the absence of any meaningful gross or microscopic changes this death is considered incidental. Otherwise, there were no test substance-related clinical observations. No statistically significant differences in mean body weights and body weight gains were observed between the test groups and the control group. Feed consumption was significantly higher in males of the high dose group during several time periods, which correlates with a slightly (not significantly) higher mean body weight in this group. There were no statistically significant differences in serum chemistry, haematology and coagulation parameters. Urinalysis showed a significantly higher urobilinogen level in low dose females, which was considered unrelated to treatment, as there was no difference in the high dose group and the difference could have been influenced by low urine volume in some animals. Macroscopic examinations at necropsy revealed no changes attributed to administration of the test material, and there were no statistically significant differences in organ weights. Histopathological examinations showed no relevant differences in the incidence and severity of findings between the high dose and the control group.

The EFSA GMO Panel concludes that there are no indications of adverse effects in this sub-chronic feeding study, in which rats were supplied diets that contained 15 % processed MON 87769 defatted soybean meal (equivalent to approximately 10.9 g/kg bw per day for males and 12.6 g/kg bw per day for females).

5.1.3.2. Twenty-eight-day repeated dose toxicity study with soybean oil[42]

A 28-day repeated dose oral study (adapted from OECD Guideline 407) was conducted to evaluate the toxicity in rats of SDA soybean oil containing 20 % SDA. Male and female Sprague–Dawley Crl:CD rats (10 animals/sex/group, individually housed) were gavaged daily for four weeks with (1) 3.0 ml/kg bw control soybean oil; or (2) 0.3 ml/kg bw SDA soybean oil mixed with 2.7 ml/kg bw control soybean oil; or (3) 1 ml/kg bw SDA soybean oil mixed with 2.0 ml/kg bw control soybean oil; or (4) 3.0 ml/kg bw SDA soybean oil. A fifth group, not gavaged, served as an additional control group. All rats were fed a standard rodent diet. Statistical analysis (one-way ANOVA followed by Dunnett's test when $p < 0.05$) was performed on body weight, body weight gain, food consumption, clinical pathology and organ weight data. All animals survived to scheduled necropsy, and there were no test substance-related clinical observations or effects on food consumption. Mean body weight gain in females of the high dose group was statistically significantly lower than that in the control group during the first week of the study, but there were no significant differences in body weights or in total body weight gain at the end of the treatment period. No test substance-related findings were noted in the haematology, serum chemistry and urine analyses, as well as in organ weights, macroscopic and microscopic examinations. Microscopic examinations (performed on oil control and high dose SDA oil groups) revealed minimal to mild hepatocellular vacuolation in most female rats with a slightly higher incidence in the control group. This effect was considered related to oil intake and, being minimal to mild and not associated with changes in liver functional parameters, it was considered not

[41] Technical Dossier/Section 7.8.4.1 i).
[42] Additional information: 23/2/2011.

adverse. Since SDA represented around 20 % of the test substance (SDA soybean oil), it can be concluded that no SDA-related effects were seen in the rats given approximately 600 mg SDA/kg bw per day for 28 days.

5.1.3.3. Sub-chronic toxicity study and one generation reproductive toxicity study with soybean oil[43]

SDA soybean oil from MON 87769 (containing 26 % SDA) was also tested in rats in a sub-chronic toxicity study (adapted from OECD Guideline 408) combined with a one generation reproductive toxicity study (adapted from OECD Guideline 415).

In the sub-chronic study, groups of 20 female Sprague–Dawley Crl:CD rats were offered a diet containing (1) SDA soybean oil at a target dose of 1.5 g/kg bw per day, supplemented with control soybean oil in order to have a total oil exposure of 4 g/kg bw per day; or (2) SDA soybean oil at a target dose of 4.0 g/kg bw per day; or (3) control soybean oil from the conventional counterpart A3525 at a target dose of 4.0 g/kg bw per day. An additional control group received a diet containing menhaden fish oil (an EPA rich and DHA rich oil) at the target dose of 4.0 g/kg bw per day. This group was included as reference control, as menhaden fish oil contains long chain omega-3 fatty acids at similar amounts as the test material (13 % EPA and 11 % DHA or 26 % SDA, respectively) and the intake of long chain omega-3 fatty acids has been associated with changes in certain clinical parameters in rats (Kroes et al., 2003; Lina et al., 2006; Blum et al., 2007; Hammond et al., 2008). The target was to constantly provide 4 g oil/kg bw per day. Statistical analysis comparing the control and SDA soybean oil treated groups included a chi-squared test for parental mating, fertility, copulation and conception indices; one-way ANOVA followed by a Dunnett's test (if $p < 0.05$) for parental and offspring body weights and body weight changes, parental food consumption and food efficiency data, oestrous cycle lengths, pre-coital intervals, gestation lengths, implantation sites, live litter sizes, unaccounted-for sites, numbers of pups born, organ weights, clinical pathology and urinalysis data; Kruskal–Wallis non-parametric ANOVA test followed by a Dunnett's test (if $p < 0.05$) on mean litter proportions of postnatal pup survival and pup sexes at birth; and Fisher's exact test on histopathological findings. There was no mortality during the treatment period and no relevant clinical findings were noted in the regular observations of the animals. Body weight and body weight gain, food consumption and food efficiency in the two groups receiving SDA soybean oil and both control groups were comparable. Haematology analysis (on 10 animals/group) showed statistically significantly higher mean absolute and per cent basophil counts in the high dose group. The absolute basophil counts were similar in the low dose group and the menhaden fish oil control group, and all values were within the ranges of the historical controls of the testing facility. At clinical chemistry analysis (conducted on 10 animals/group) a reduction in mean cholesterol level was noted in the group administered menhaden fish oil, in the low dose group (not statistically significant in both) and in the high dose group (statistically significant). These changes are not regarded as indications of adverse effects. Urinalysis revealed no relevant findings. Absolute and relative weights and macroscopic appearance of organs and tissues at necropsy did not differ between the groups administered SDA soybean oil and the control soybean oil, and microscopic examinations of selected organs and tissues showed no relevant differences in histopathological changes. No SDA soybean oil-related effects were observed at doses as high as 4 g SDA oil/kg bw per day.

In the one-generation reproductive toxicity study, the same test and control materials were administered in the diet at the same dose levels to groups of 25 male and 25 female animals. The F_0 males were treated for at least 70 days prior to mating and afterwards until euthanasia (for a total of 127–129 consecutive days). The F_0 females were also treated for at least 70 days before mating and during gestation and lactation (for a total of 113–127 consecutive days). The F_1 progeny was assumed to be exposed *in utero* during gestation and throughout lactation. Dams and F_1 progeny were sacrificed at weaning on postnatal day 21.

During the treatment period there was no test substance-related mortality, and no clinically relevant effects were noted. Body weights and body weight gains, food consumption and food efficiency in the

[43] Additional information: 23/2/2011.

two dose groups receiving SDA soybean oil were comparable to those in the soybean oil control group. Haematology, clinical chemistry and urine analyses were performed only on F_0 males (10 animals per group) just prior to the scheduled necropsy. In the high dose group a statistically significantly reduced mean serum triglyceride level was observed. The level was also reduced in the low dose group (not significant) and in the menhaden fish oil group. Animals of the high dose group also showed lower cholesterol levels (not significant) than the soybean oil control group. Similar changes in serum lipid levels were also observed in other studies. They are attributable to the administration of high levels of long chain omega-3 fatty acids and not considered adverse. Other statistically significant differences in relation to the control group (related to blood urea nitrogen and phosphorus levels in clinical chemistry analysis as well as urobilinogen concentration in urinalysis) are regarded as incidental findings since the differences were small and/or not dose related. Organ weight determinations (20 animals/sex) showed no statistically significant differences between the groups administered SDA soybean oil and the group given the control soybean oil, and no relevant differences between these groups were noted in the macroscopic examinations performed at necropsy. In the microscopic examinations of selected organs and tissues a thyroid gland follicular adenoma was found in a male rat of the high dose SDA soybean oil group. This type of lesion was reported to be common in the historical control data. As no pre-neoplastic changes were identified in the thyroid gland of the other high dose animals, this tumour was considered to be of spontaneous and incidental occurrence and not related to the treatment. Regarding the other organs and tissues, no relevant differences in the incidence and severity of histological findings were identified between groups.

The study included an evaluation of potential effects of the test material on male and female reproductive processes including gonadal function, oestrous cycle, mating behaviour, conception, gestation, parturition and lactation and on the growth and development of the offspring through weaning. No test substance-related effects on any of the fertility and reproductive performance parameters were observed. Regarding the F_1 progeny, there was no difference between the groups receiving SDA soybean oil and the control soybean oil regarding litter size and pup survival. Clinical observations on pups and observations made at necropsy were unaffected by dietary test substance exposure, were similar across all groups and were within the normal ranges for pups of this age. Body weights on the first day after birth were slightly but statistically significantly lower in males of the low dose group and females of the low dose and high dose groups than in the control group. As differences were small, weights were comparable to those in pups of menhaden-treated rats and within the historical range of the test laboratory, and no difference were noted later in the study, the EFSA GMO Panel considers these observations not to raise safety concerns.

It is concluded that in the sub-chronic toxicity study and the one generation reproductive toxicity study administration of SDA soybean oil at the high dose level (4.0 g SDA soybean oil/kg bw per day corresponding to approximately 1.0 g SDA/kg bw per day) did not induce toxicologically relevant effects when compared with conventional soybean oil and menhaden fish oil.

5.1.3.4. Chicken feeding study with defatted soybean meal[44]

A 42-day feeding study in broiler chickens (*Gallus domesticus*) was provided. A total of 960 Ross × Ross 308 day-old broiler chicks were randomly allocated to eight groups, each group consisting of 120 broilers housed in 10 pens (12 birds per pen, five pens per sex). On day 7, all pens were adjusted to 10 birds according to a well-described procedure. Two groups received diets containing defatted soybean meal derived from MON 87769 (test group, verified by PCR) or the conventional counterpart (control group: A3525). The other six groups received diets containing defatted soybean meal derived from a non-GM commercial varieties (P93B87, H3395, NK32Z3, Midwest 3444, 93B15, PN93B82). Before mixing, all soybean varieties were identified by PCR and analysed for nutrients, anti-nutrients, relevant mycotoxins and pesticides. Maize and corn gluten meal were analysed for protein, moisture and amino acids prior to diet formulation. Isocaloric diets consisting mainly soybean, maize and soybean oil were formulated to meet the nutrient requirements (NRC, 1994), which was confirmed by a compositional analysis.

[44] Dossier: Part I—Section D 7.10.2 and MSL 21498 (2008). Additional information: 1/10/2010.

Each group of birds was fed *ad libitum* with starter (day 0–21) and grower/finisher (day 22–42) diets containing approximately 35 % or 31 % soybean meal, respectively. Birds were observed twice daily for clinical signs. All birds were weighed by pen at the end of the study (day 42). Two broilers per pen were slaughtered on day 43 or day 44 for measurement of body composition.

The EFSA GMO Panel notes the high animal losses over the 42 days of the study—on average 10.9 % in the eight treatment groups; 10.3 % in the group given a diet with soybean MON 87769. Mortality in the first week was attributed predominantly to bacterial infections and dehydration. No conclusion can be derived from this study, owing to the high mortality.

5.1.4. Allergenicity

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

5.1.4.1. Assessment of allergenicity of the newly expressed proteins

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

The *PjΔ6D* and *NcΔ15D* genes originate from *Primula juliae* and *Neurospora crassa*, respectively, which are not considered to be common allergenic sources. Some species of *Primula* are known to give rise to contact dermatitis, but this has not been reported for *P. juliae* and is primarily due to benzoquinones and related compounds in the plant (e.g. primin = 2-methoxy-6-pentylbenzoquinone) (Horper and Marner, 1996; Aplin and Lovell, 2001).

A bioinformatics-supported comparison of the amino acid sequences of the PjΔ6D and NcΔ15D proteins using the criterion of 35 % identity in a window of 80 amino acids revealed no significant similarities to known allergens.[45] In addition, the applicant also performed analyses searching for matches of eight contiguous identical amino acid sequences between the PjΔ6D and NcΔ15D proteins and known allergens. One match of eight contiguous serine amino acids (SSSSSSSS) was identified when the NcΔ15D query sequence was used. This portion of the query protein aligned with a sequence of nine consecutive serine residues in *Triticum aestivum* serine carboxypeptidase. Such stretches of contiguous serines are present in many non-allergenic proteins. No evidence has been found that eight contiguous serine residues indicate a shared unique allergen epitope sequence. This identical match did not raise concern for the EFSA GMO Panel.

Studies on resistance to degradation of the PjΔ6D and NcΔ15D proteins have been described in Section 5.1.2.2.

In the context of the present application, the EFSA GMO Panel considers that there are no indications that the newly expressed PjΔ6D and NcΔ15D proteins in soybean MON 87769 may be allergenic.

5.1.4.2. Assessment of allergenicity of the GM plant[46]

Soybean is considered to be a common allergenic food.[47] The applicant performed *in vitro* allergenicity studies with extracts of soybeans MON 87769, its conventional counterpart (A3525) and different non-GM reference soybean varieties. The IgE-binding capacity of soybean proteins to sera from 16 individuals clinically documented to be allergic to soybean and six non-allergic individuals

[45] Technical Dossier/Section 7.9.1 iii). Additional information: 17/5/2013.
[46] Dossier: Part I—Section 7.9.2. Additional information: 10/11/2011.
[47] 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, p. 11–14.

were quantified using an enzyme-linked immunosorbent assay (ELISA) to investigate whether the allergenicity potential of soybean MON 87769 is altered in comparison with its conventional counterpart and non-GM reference varieties. The sera from allergic individuals had similar reactivity to proteins in extracts from soybean MON 87769 and the conventional counterpart.

The applicant performed two-dimensional (2D) electrophoresis of extracts of soybean MON 87769 and its conventional counterpart followed by Western blotting using individual sera from two allergic humans to soybean. This study showed no meaningful differences in the IgE-binding patterns between the extracts of proteins derived from soybean MON 87769 and its conventional counterpart. Owing to the limitations associated with the low number of allergic individuals used, this information was used as supplementary information of the quantitative ELISA study described above.

The applicant also performed a one-dimensional (1D) Western blot analysis using pooled sera from individuals allergic to soybean. The EFSA GMO Panel has previously presented the limitations of the 1D-PAGE gels and the use of pooled sera for the allergenicity assessment (see Annex 4 and Annex 5 of EFSA, 2010a).

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

5.1.5. Nutritional assessment of GM food/feed

This assessment focuses on SDA as the most significant modification in MON 87769 soybean oil, and on the consequences of the reduction in the level of the essential fatty acid linoleic acid.

5.1.5.1. Human nutritional assessment

SDA, a (n-3) long-chain PUFA, is a metabolic intermediate in the conversion of ALA to EPA and DHA (Whelan and Rust, 2006). ALA is poorly converted to EPA in humans (Burdge and Calder, 2005). SDA, however, appears to be more readily metabolised than ALA to EPA with a conversion efficiency between 3:1 and 6:1 (SDA:EPA) (James et al., 2003; Harris et al., 2008; Lemke et al., 2010). This means that an intake of around 750 mg SDA/day could theoretically have the same effect as an intake of 125–250 mg EPA/day.

The ability of SDA to increase EPA in blood is higher than that of ALA. James et al. (2003) noted that 4.2 g SDA/day increased the omega-3 index (sum of EPA plus DHA as a percentage of total fatty acids) in red blood cell lipids to a degree similar to 1 g EPA/day.

Unlike ALA, SDA is not an essential fatty acid, and no dietary reference value has been defined. For EPA and DHA, EFSA (2010b) has set an adequate intake (AI) level of 250 mg EPA + DHA/day for adults, based on considerations of cardiovascular health.

(a) Replacement of soybean oil with the MON 87769 oil in targeted use

According to the applicant, SDA soybean oil is intended for use in a wide variety of food products at levels that will provide 375 mg SDA per serving.[48] Four servings/day would provide 1 500 mg SDA.

Using information from the United Kingdom (UK) National Diet and Nutrition Survey (adults 19–64 years old)[49] and the US FDA information on serving sizes, the applicant calculated the intake of SDA-rich soybean oil and SDA. These calculations also allow estimations of altered intakes of other fatty acids in the diet. By assuming that all foods proposed would use the MON 87769 oil (containing 20 % SDA) as an ingredient in the recipe, the average total intake of SDA soybean oil for the UK adult population would be 11.8 g/day (90th percentile 18.5 g/day, 97.5th percentile 24.0 g/day).[50] This intake

[48] Dossier: Part I—Section D7.7 and D7.10.1.
[49] Dossier: Part I—Section 7.10.1 and Wang and Petersen (2009).
[50] Dossier: Part I—Section D7.10.1.

corresponds to 0.16 g SDA soybean oil/kg bw/day (90th and 97.5th percentile 0.25 and 0.32 g/kg bw/day, respectively). Assuming an SDA content of 20.7 % of the total fatty acids in the SDA soybean oil, the estimated mean per capita intake of SDA from the suggested uses would be 2.5 g/day (3.8 and 5.0 g/day for the 90th and 97.5th percentile, respectively). On a body weight basis, these intakes of SDA correspond to 33 mg/kg/day (63.7 mg/kg bw/day at the 97.5th percentile). Because of higher calculated intakes of soybean oil in men, the mean SDA intake would be 2.8 g/day (5.6 g/day at the 97.5th percentile) for men and 2.1 g/day for women (4.0 g/day at the 97.5th percentile). The baseline intake of SDA from seafood in the UK is estimated to be around 20 mg/day.[51]

Using the enrichment of red blood cell membranes with EPA as a marker and noting that SDA was 17–33 % as effective as EPA itself in increasing the level of EPA, with negligible conversion to DHA (James et al., 2003; Harris et al., 2008), the applicant calculated that the estimated mean per capita intake of SDA from the suggested use of SDA soybean oil (2.5 g SDA/person/day), would be equivalent to a dietary intake of around 0.4–0.8 g EPA/person/day (1.0–1.8 g/person/day for the 97.5th percentile). Adding this calculated estimate of dietary EPA obtained from SDA soybean oil to the background intake of DHA and EPA in the UK (0.15 g/person/day) would result in a cumulative estimated intake below the level of 5 g/day of supplemental combinations of EPA and DHA and of 1.8 g of EPA alone per day, which were considered to be safe for adults by EFSA (2012).

(b) Replacement of vegetable oil with the MON 87769 oil in foods

The applicant also used the UK National Diet and Nutrition Survey to estimate the impact of replacing presently used vegetable oils in foods with SDA-rich soybean oil on the intake of other fatty acids. Under this scenario the intake of oleic acid would be reduced by 2.39–3.02 g/day (range for SDA-rich soybean oil containing 30 % and 20 % SDA, respectively), and LA intake reduced by 1.47–1.38 g/day. The 2.44 g/day intake of SDA would be accompanied by an increased intake of GLA of 0.62–0.76 g/day, of palmitic acid by 0.42–0.56 g/day, of ALA of 0.26–0.41 g/day and of stearic acid of 0.12–0.22 g/day. Thus, the dietary intake of n-3 PUFAs would increase by 2.70–2.85 g/day, whereas the intake of n-6 PUFAs would decrease by 0.85–0.62 g/day. The total saturated fatty acid intake would increase by 0.54–0.79 g/day.

With regard to the AI for LA established by EFSA (2010b) and corresponding to 4 E% (9 g/day with a 2000 kcal diet) and observed intakes in European countries between 3.7 and 5.8 E% (7.8–18.6 g/day), the estimated reduction in LA intake is without concern. The estimated increase of ALA with the use of SDA soybean oil can be considered to be desirable, in view of an adequate intake of 0.5 E% (1.1 g/day with a 2000 kcal diet) and an observed intake of ALA of 0.4–0.8 E% (0.7–2.3 g/day). The increase in γ-linolenic and saturated fatty acids intake is also without concern.

An overall increase in the dietary intake of total trans-fatty acids is not to be expected because (i) only a very small amount of trans-SDA is present in the refined bleached and deodorised oil from soybean MON 87769, and (ii) the total trans-fatty acid content in MON 87769 soybean oil similar to that of conventional soybean oils (typically < 2% of total fat). Therefore, no adverse effects of these trans-fatty acids are expected when conventional vegetable oils are replaced by MON 87769 soybean oil.

(c) Replacement of soybean with the MON 87769 in foods

Upon request, the applicant performed an additional assessment of the changes in fatty acid intake of consumers owing to substitution of conventional soybeans in soybean foods including soybean oil, with soybeans MON 87769.[52] The consumption data of such foods were taken from the EFSA Comprehensive European Food Consumption Database. UK and France were chosen as examples of relatively high consumption countries and Denmark as an example of a country with low consumption of soybean derived foods. The mean, lower and upper limit values of fat coming from soybean ingredients for the foods of the Comprehensive Database were provided for Denmark (Danish Food Composition Databank, Version 7.01, March 2009), France (French food composition table CIQUAL,

[51] Dossier: Part I—Section D7.10.1.
[52] Additional information: 20/9/2012 and 18/2/2013.

2008) and the UK (McCance and Widdowson's Composition of Foods Integrated Dataset, 2002).[53] In each case the highest reported fat or fatty acid content value reported was used in the calculation.

The relative changes in fatty acid intakes of adults using soybean MON 87769 ingredients instead of conventional soybean ingredients for these three countries were calculated. The greatest changes occurred in the UK and consisted of an increase in the ALA intake of 0.5 g/day, in the SDA intake of 3.4 g/day, in the GLA intake of 1.1 g/day and in the palmitic acid intake of 0.17 g/day, whilst the intake of LA decreased by 4.9 g/day and that of oleic acid by 0.5 g/day.

The EFSA GMO Panel considers only the change in the intake of SDA and LA to be of nutritional importance. There is one study on the consumption of 4.2 g of SDA over a period of 12 weeks, indicating no adverse effects of SDA at such high doses (Lemke et al., 2010). The decrease in LA intake of 4.9 g/day would result in a LA intake of 6.5 g/day in an average UK consumer of soybean foods. This LA intake would correspond to about 3 E%, which is below the AI (EFSA, 2010b). According to EFSA, the mean LA intake in four EU countries ranged from 7.8 to 18.6 g/day and the average intake of cis n-6 PUFA was between 3.8 E% and nearly 6 E%. Distribution of intakes were available only for the Netherlands, ranging from 2.6 to 9.8 E% at the 5th and the 95th percentile, respectively, and for the UK, ranging from 1.9 to 10.5 E% at the 2.5th and 97.5th percentile, respectively (EFSA, 2010b).

In the view of the Panel this is a very conservative estimate with a high likelihood of an overestimation of changes in fatty acid intake, because all subjects were assumed to consume daily all of the included soybean-containing foods, with the highest reported fat content, and the use of soybean MON 87769 and of its oil in the manufacture of all foods containing soybean ingredients. In consideration of this overestimation of potential changes in fatty acid intake, the EFSA GMO Panel concludes that the estimated decrease in the LA intake of adults is not of safety concern. Because of the lack of consumption data, the applicant could not provide a similar estimate for young children.

5.1.5.2. Animal nutritional assessment[54]

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, improve the quality/stability of pellets and add energy to the diets. Defatted toasted soybean meal represents the most common soybean by-product used in zootechnical animal feed formulations, with around 90 % of the defatted soybean meal entering the feed chain in the EU, mainly given to poultry, pigs and cattle. Since the compositional analysis in defatted and toasted meal, protein isolate and crude lecithin produced from soybean MON 87769 and from its conventional counterpart were similar, the EFSA GMO Panel is of the opinion that the incorporation of feeding stuff derived from soybean MON 87769 in nutritionally balanced diets has no impact on health and performance of the tested species.

Although the applicant considers the use of oil from MON 87769 in animal feed unlikely in the absence of a specific claim for modified product quality, reference is made to a publication (Rymer et al., 2011) which considers the impact of an SDA rich diet in chickens. In this study the effect of feeding broiler chickens diets containing modest amounts (4.5–5.0 % of the feed) of conventional soybean oil, oil from soybean MON 87769 (24.1 % stearidonic acid) or fish oil (1.4 % stearidonic acid) were investigated. This was done to assess the fatty acid composition of the edible tissues and the sensory characteristics of the chicken meat. Birds fed the SDA diet had performance comparable to that of broiler chickens supplied the control diet (feed intake, weight gain, feed to gain ratio and yield of meat). However, the SDA diet resulted in increased total n-3 fatty acids in skinless breast and leg meat and reduced total n-6 fatty acids in skinless breast meat. Broiler chickens fed diets supplemented with the SDA-containing soybean oil produced meat with increased SDA concentration, EPA and

[53] Additional information: 18/2/2013 (Table 2).
[54] Dossier: Part I—Sections 7.8.3 and 7.10.2.

DPA, but not DHA (except in the skinless breast meat). There was no evidence that the broiler chickens converted SDA to long chain n-3 PUFAs any more efficiently than C18:3 n-3 linolenic acid.

In a small study, Bernal-Santos et al. (2010) investigated if dairy cows supplied with SDA soybean oil would produce milk with enhanced omega-3 fatty acids. The study indicated that rumen-protected formulations of SDA soybean oil would be needed to increase the n-3 fatty acid content of milk fat.

The EFSA GMO Panel concludes that feeding of full-fat soybean MON 87769 or inclusion of the oil derived from MON 87769 could alter the lipid content of animal tissues.

However, the Panel did not consider the nutritional impact by consuming products of animal origin derived from animals fed whole fat MON 87769 or its oil on consumers.

5.1.5.3. Conclusion

The EFSA GMO Panel concludes that the proposed uses of MON 87769 soybean oil in foods will not result in intakes of SDA with adverse effects and that the other changes in the dietary fatty acid pattern are unlikely to have negative nutritional consequences for human. The EFSA GMO Panel notes that the quantitative dietary estimates described here would have to be revisited if the oil produced by soybean MON 87769 were to be extensively used in food products not considered in this assessment, for example as dietary supplements or to modify animal feed products.

5.1.6. Post-market monitoring of GM food/feed

EFSA recommends that a proposal for a post-market monitoring (PMM) plan should be provided by the applicant (EFSA, 2006, 2011). EFSA recommends that the PMM plan should include the collection of consumption data for the European population.

5.1.7. Scientific correctness of proposed labelling

Considering the altered composition and nutritional values of soybean MON 87769, the EFSA GMO Panel considered a specific labelling proposal provided by the applicant in accordance with Articles 13(2)(a) and 25(2)(c) of Regulation (EC) No 1829/2003. The applicant proposed that food and feed products within the scope of the application should be labelled as "genetically modified soybean containing SDA omega-3 oil" or "contains genetically modified soybean containing SDA omega-3 oil". The GMO Panel is of the opinion that the compositional data (see Section 4.1.3) show that the fatty acid composition of seeds of soybean MON 87769 and derived oil has indeed been changed in relation to the conventional counterpart.

The EFSA GMO panel considered the proposed labelling and confirms its scientific correctness.

5.2. Conclusion

No relevant similarities to known toxic proteins and allergens were found for *Primula juliae* Δ6 and *Neurospora crassa* Δ15 desaturases. Based on a weight-of-evidence approach, the Panel concludes that there are no reasons to suppose that these specific desaturases would introduce safety concerns.

Testing of extracts from soybeans MON 87769 with sera from individuals allergic to soybean showed that the overall allergenicity of the whole plant had not been changed.

In toxicological studies, no SDA soybean oil-related effects were observed at doses as high as 1 000 mg SDA/kg bw/day. Published literature provides ample evidence that an increased exposure to GLA would not raise safety concerns.

The EFSA GMO Panel concludes that the estimated changes in fatty acid intake by consumers following the use of oil from soybean MON 87769 are unlikely to constitute a toxicological risk or to have negative nutritional consequences for humans.

Nutritional equivalence indicated by the compositional analysis was further supported by the outcome of feeding study in rat. The EFSA GMO Panel is of the opinion that feeding stuffs derived from defatted soybean MON 87769 are as safe and nutritious as those derived from other non-GM soybean varieties.

EFSA recommends that a proposal for a PMM plan be provided by the applicant. EFSA recommends that the PMM should include the collection of consumption data for the European population.

6. Environmental risk assessment and monitoring plan

6.1. Evaluation of relevant scientific data

Considering the scope of application EFSA-GMO-UK-2009-76, the environmental risk assessment (ERA) of soybean MON 87769 is concerned with (i) exposure of bacteria to recombinant DNA in the gastrointestinal tract of animal fed GM material and those present in environments exposed to faecal material; and (ii) accidental release into the environment of viable seeds of soybean MON 87769 during transportation and processing. Moreover, in terms of environmental exposure, the applicant indicates that soybean MON 87769 will be processed in dedicated facilities in the countries of production and that it is therefore not expected that large quantities of viable soybean MON 87769 seeds will be exported to the EU.

6.1.1.1. Potential unintended effects on plant fitness owing to the genetic modification[55]

Cultivated soybean (*Glycine max* (L.) Merr.) is a species in the sub-genus *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,[56] 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 only under certain environmental conditions grow as volunteers in the year following cultivation. 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 out-of-season germination resulting in death, or owing to management practices prior to planting the subsequent crop (Owen, 2005).

The applicant provided agronomic and phenotypic data on soybean MON 87769 from field trials carried out in the USA in 2006 and 2007 (see Section 4.1). Eighteen agronomic and phenotypic characteristics were measured and possible interactions with biotic (e.g. disease damage, arthropod damage and abundance) and abiotic factors were also assessed in the same trials.

Here, special attention is paid to any agronomic and phenotypic characteristics which may affect fitness characters (e.g. survival, establishment and spread) of soybean MON 87769 seeds which could be accidentally released into the environment, for example plant stand, yield, plant height, seedling vigour, germination and dormancy. There were no significant differences across field trials or laboratory experiments. Some site-specific significant differences were observed, but they were not indicative of a consistent plant response associated with the trait and, in most cases, they suggest a lower fitness of soybean MON 87769 (e.g. lower germination capability or lower yield).

Furthermore, there is no evidence that the expected changes in seed fatty acid composition would confer a potential selective agronomic advantage to the GM soybean compared with its conventional counterpart. However, survival of soybean plants outside of cultivation or other areas is mainly limited by a combination of low competitiveness, absence of a dormancy phase, and susceptibility to plant pathogens and cold climate conditions. As these general characteristics are unchanged in soybean

[55] Technical dossier/Section 9.1.
[56] Available online: http://epp.eurostat.ec.europa.eu/portal/page/portal/agriculture/data/database

MON 87769, it can be considered that soybean MON 87769 has no altered survival, multiplication or dissemination characteristics compared with its conventional counterparts.

In addition to the data presented by the applicant, the EFSA GMO Panel is not aware of any scientific report of increased spread and establishment of existing GM soybeans and any change in survival capacity, including overwintering (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 unintended environmental effects of soybean MON 87769 in Europe will not be different to that of conventional soybean varieties.

6.1.1.2. Potential for gene transfer[57]

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 vertical gene flow via seed dispersal and cross-pollination.

(a) Plant to bacteria gene transfer

Genomic plant DNA is a component of several food and feed products derived from soybean. It is well documented that DNA present in food and feed becomes substantially degraded during processing and digestion in the human or animal gastrointestinal tract. However, a low level of exposure of fragments of ingested DNA, including the recombinant fraction of such DNA, to microorganisms in the digestive tract of humans, domesticated animals and other environments exposed to the GM plant or plant material is expected.

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

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

The presence of DNA sequence similarity between the recombinant desaturase genes in soybean MON 87769 and other naturally occurring desaturase genes is possible. However, as discussed further below, no selective advantage of hypothesised transformed cells is predicted. The flanking regions of the recombinant gene insert contain approximately 40- and 270-bp-long sequences of the truncated right and left border of the Ti-plasmid of *Agrobacterium tumefaciens*. Thus, there is only a limited capacity to facilitate horizontal gene transfer by homologous recombination. *A. tumefaciens* occurs in soil and is not considered to be prevalent in the main receiving environment (i.e. the gastrointestinal tract of humans or animals). However, occurrence of the recombinant genes outside their immediate receiving environment in the habitats of both bacterial species cannot be ruled out (Hart et al., 2009) and is therefore also considered here.

On a theoretical basis (i.e. without any study providing experimental evidence for HGT in the case of GM food and feed derived from soybean MON 87769 or any other GM plant), it can be assumed that, as a rare event, homologous recombination can occur between the recombinant desaturase gene and other desaturase genes present in the environment. Such recombination events would only replace

[57] Technical dossier/Section D6.

natural variants (i.e. substitutive recombination) and are therefore are unlikely to provide any new property connected to a selective advantage for the recipient organisms (EFSA, 2009). Double homologous recombination of the flanking regions with those on Ti-plasmids of *A. tumefaciens* would result in gene replacement, by which the Δ6 and Δ15 desaturase genes would substitute genes for crown gall formation (loss of auxin-, cytokinin- and opine-synthesising genes).

In addition to homology-based recombination processes, illegitimate recombination that does not require DNA similarity between the recombining DNA molecules is theoretically possible. However, the transformation rates for illegitimate recombination are considered to be 10^{10}-fold lower than for homologous recombination (Hülter and Wackernagel, 2008; EFSA, 2009). Illegitimate recombination events have not been detected in studies that have exposed bacteria to high concentrations of GM plant DNA (EFSA, 2009) and the process is therefore not considered further in the assessment of plant to bacterial gene transfer scenarios.

Both desaturase genes are under the control of a seed specific promoter. The expression of such promoter–gene constructs in bacteria is unknown, but is generally assumed to be low (Warren et al., 2008).

In a worst case scenario, considering the possibility of expression, an *A. tumefaciens* recipient would become capable of producing Δ6 and Δ15 desaturases. However, the exposure of bacterial communities to the soybean MON 87769 *Pj.D6D* and *Nc.Fad3* genes must be seen in the context of the natural occurrence and level of exposure to alternative sources of genetically diverse Δ6 and Δ15 desaturase genes to which bacterial communities are continually exposed. Desaturases are widely abundant in all organisms, including bacteria, fungi, plants and animals, where they play a key role in the maintenance of the proper structure and functioning of biological membranes (Los and Murata, 1998). In plants and bacteria, desaturases can influence the fluidity of the cytoplasmic membrane by increasing the level of unsaturated fatty acids in the phospholipids (Mikami and Murata, 2003). In microorganisms, stress factors such as cold temperatures, gamma-radiation or antimicrobial agents (chitosan) can induce the activity of desaturases, providing a mechanism for adaptation (Dussault et al., 2009; Palma-Guerrero et al., 2010; Shivaji and Prakash, 2010). Owing to its specific lifestyle as a soil bacterium and plant pathogen, the EFSA GMO Panel considers it unlikely that *A. tumefaciens* would gain selective advantage from such a HGT by double homologous recombination.

The EFSA GMO Panel concludes that the desaturase genes from soybean MON 87769 could, on a theoretical basis, be transferred on extremely rare occasions by double homologous recombination to *A. tumefaciens*. However, since *A. tumefaciens* is not a member of the gut microbiota, exposure to recombinant DNA of MON 87769 is considered to be very low. Owing to the natural occurrence of desaturases in the environment, a low-level gene transfer to *A. tumefaciens* is not seen to confer a novel selective advantage. Considering its intended use as food and feed and the above assessment, the EFSA GMO Panel has therefore not identified a concern associated with HGT from soybean MON 87769 to bacteria.

(b) Plant to plant gene transfer

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

The genus *Glycine* is divided into two distinct sub-genera: *Glycine* and *Soja*. Soybean is in the subgenus *Soja*. The subgenus *Glycine* contains 16 perennial wild species, while the cultivated soybean, *G. 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 only with other members of *Glycine* sub-genus *Soja* (Hymowitz et al., 1998; Lu, 2005). Hence, the three species of the sub-genus *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). However, 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 the occasional soybean plants resulting from seed spillage in the EU.

Soybean is an annual almost completely self-pollinating crop in the field, which has a percentage of cross-pollination usually lower than 1 % (Weber and Hanson, 1961; Caviness, 1966; 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).

Plant to plant gene flow could therefore occur under the following scenario: imports of soybean MON 87769 seeds (while most MON 87769 seeds will be processed in countries of production), processing outside importing ports, transport in regions of soybean production in Europe, spillage of GM seeds during transport, germination and development of spilled seeds within soybean fields or in the very close vicinity of cultivated soybean fields, overlap of flowering periods and particular environmental conditions favouring cross-pollination. The overall likelihood of cross-pollination between GM soybean plants and cultivated soybean is therefore extremely low. Apart from seed production areas, such plants will not persist over time. Dispersal of soybean seeds by animals is not expected owing to the characteristics of the seed, but accidental release into the environment of seeds may occur during transport and processing for food, feed and industrial uses. However, cultivated soybean seeds rarely display any dormancy characteristics and only under certain environmental conditions grow as volunteers in the year following cultivation (OECD, 2000). Even in soybean fields, seeds usually do not survive during the winter owing to herbivory, rotting, out-of-season germination resulting in death, or as a result of management practices prior to planting the subsequent crop (Owen, 2005).

The EFSA GMO Panel takes into account that this application does not include cultivation of the soybean within the EU so that the likelihood of cross-pollination between cultivated soybean and the occasional soybean plants resulting from seed spillage is considered extremely low. However, in countries cultivating this GM soybean and producing seed for export, there is a potential for admixture in seed production and thus the introduction of GM seeds through this route. Hence, it is important that appropriate management systems are in place to restrict seeds of soybean MON 87769 entering cultivation as this would require specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

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

6.1.1.3. Potential interactions of the GM plant with target organisms[58]

Owing to the type of trait (i.e. changes in the fatty acid profile) and the scope of application EFSA-GMO-UK-2009-76, this was not considered a relevant issue by the EFSA GMO Panel.

[58] Technical dossier/Section 9.4.

6.1.1.4. Potential interactions of the GM plant with non-target organisms[59]

Owing to the scope of application EFSA-GMO-UK-2009-76, 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.

6.1.1.5. Potential interactions with the abiotic environment and biogeochemical cycles[60]

Owing to the scope of application EFSA-GMO-UK-2009-76, 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.

6.1.2. Post-market environmental monitoring[61]

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 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 the PMEM plan provided by the applicant (EFSA, 2011). The potential exposure to the environment of soybean MON 87769 would be through faecal material from animals fed soybean MON 87769 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 methods of transportation, accidental spillage cannot be excluded. Hence, it is important that appropriate management systems are in place to restrict seeds of soybean MON 87769 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 an approach involving operators (federations involved in soybean import and processing) reporting to the applicant via a centralised system any observed adverse effect(s) of GMOs on human health and the environment; (2) a coordinating system established by EuropaBio for the collection of the information recorded by the various operators; and (3) the use of networks of existing surveillance systems (Lecoq et al., 2007; Windels et al., 2008). The applicant proposes to submit a PMEM report on an annual basis and a final report at the end of the consent.

The EFSA GMO Panel is of the opinion that the scope of the PMEM plan proposed by the applicant is in line with the intended uses of soybean MON 87769 as the ERA did not cover cultivation and identified no potential adverse environmental effects. No case-specific monitoring is necessary. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

6.2. Conclusion

Considering the scope of application EFSA-GMO-UK-2009-76, there are no indications of an increased likelihood of establishment and spread of feral soybean MON 87769 plants in the case of accidental release into the environment of viable GM soybean seeds. The low levels of environmental exposure of these GM soybean plants indicate that the risk to non-target organisms is extremely low. The theoretically possible transfer of the recombinant genes from soybean MON 87769 to environmental bacteria does not raise a concern owing to the lack of both an efficient transfer mechanism and an identified selective advantage. The scope of the PMEM plan provided by the

[59] Technical dossier/Section 9.5.
[60] Technical dossier/Sections 9.8 and D10.
[61] Technical dossier/Section 11.

applicant and the reporting intervals are in line with the intended uses of soybean MON 87769 and the guidance document. In addition, the EFSA GMO Panel acknowledges the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure in cases of accidental release of viable seeds of soybean MON 87769.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The EFSA GMO Panel was requested to carry out a scientific risk assessment of soybean MON 87769 for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003.

The molecular characterisation of soybean MON 87769 does not raise safety issues.

No differences in the agronomic and phenotypic characteristics and the composition except the intended modification of the fatty acid profile requiring further assessment were identified.

No relevant similarity to known toxic proteins or allergens was found for the newly expressed *Primula juliae* Δ6 and *Neurospora crassa* Δ15 desaturases. Based on a weight-of-evidence approach, the EFSA GMO Panel concludes that there are no reasons to suppose that these desaturases would pose safety concerns. The genetic modification is not expected to change the overall allergenicity of soybean MON 87769 when compared with that of its conventional counterpart.

Studies with rats indicate that feeding stuffs derived from soybean MON 87769 are as safe and nutritious as those derived from other non-GM soybean varieties.

Consumption of MON 87769 soybean oil replacing other oils in food is not expected to result in adverse effects from increased SDA intake as shown in different exposure scenarios. The other changes in the dietary fatty acid pattern are unlikely to have negative nutritional consequences.

Since the use of oil derived from the soybean MON 87769 will result in a higher intake of SDA, a PMM plan is recommended to confirm the exposure assessment using realistic consumption data for the European population.

Considering the scope of application EFSA-GMO-UK-2009-76, there are no indications of an increased likelihood of establishment and spread of feral soybean MON 87769 plants in the case of accidental release into the environment of viable GM soybean seeds. The low levels of environmental exposure of these GM soybean plants indicate that the risk to non-target organisms is extremely low. The theoretically possible transfer of the recombinant genes from soybean MON 87769 to environmental bacteria does not raise a concern owing to the lack of both an efficient transfer mechanism and an identified selective advantage. The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of soybean MON 87769 and the guidance document of the EFSA GMO Panel on PMEM of GM plants (EFSA, 2011). In addition, the EFSA GMO Panel acknowledges the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure in cases of accidental release of viable seeds of soybean MON 87769.

In conclusion, the EFSA GMO Panel considers that the information available for soybean MON 87769 addresses the scientific issues indicated by the Guidance document of the EFSA GMO Panel and the scientific comments raised by the Member States, and that soybean MON 87769 is as safe as its conventional counterpart and is unlikely to have adverse effects on human and animal health and the environment in the context of the scope of this application.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of the United Kingdom, received on 20 October 2009, concerning a request for the placing on the market of genetically modified soybean MON 87769 submitted under Regulation (EC) No 1829/2003 by Monsanto Europe S.A./N.V.

2. Acknowledgement letter, dated 10 November 2009, from EFSA to the Competent Authority of the United Kingdom.

3. Letter from EFSA to applicant, dated 27 November 2009, requesting additional information under completeness check.

4. Letter from applicant to EFSA, received on 26 January 2010 and 11 February 2010, providing additional information under completeness check.

5. Letter from EFSA to applicant, dated 16 February 2010, delivering the "Statement of Validity" for application EFSA-GMO-UK-2009-76, regarding genetically modified soybean MON 87769 submitted under Regulation (EC) No 1829/2003 by Monsanto Europe S.A./N.V.

6. Letter from EFSA to applicant, dated 3 May 2010, requesting additional information and stopping the clock.

7. Letter from applicant to EFSA, received on 14 June 2010, providing additional information.

8. Letter from EFSA to applicant, dated 5 August 2010, requesting additional information and maintaining the clock stopped.

9. Letter from applicant to EFSA, received on 1 October 2010, providing additional information.

10. Letter from EFSA to applicant, dated 13 October 2010, requesting additional information and maintaining the clock stopped.

11. Letter from applicant to EFSA, received on 24 February 2011, providing additional information.

12. Letter from EFSA to applicant, dated 20 July 2011, requesting additional information and maintaining the clock stopped.

13. Letter from applicant to EFSA, received on 10 November 2011, providing additional information.

14. Letter from EFSA to applicant, dated 7 February 2012, requesting additional information and maintaining the clock stopped.

15. Letter from applicant to EFSA, received on 20 September 2012, providing additional information.

16. Letter from EFSA to applicant, dated 9 January 2013, requesting additional information and maintaining the clock stopped.

17. Letter from applicant to EFSA, received on 18 February 2013, providing additional information.

18. Letter from applicant to EFSA, received on 21 May 2013, providing additional information.

19. Letter from EFSA to applicant, dated 10 October 2013, requesting additional information and maintaining the clock stopped.

20. Letter from applicant to EFSA, received on 3 January 2014, providing additional information.

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