European Food Safety Authority

EFSA Journal 2012;10(10):2909

## SCIENTIFIC OPINION

# Scientific Opinion on application (EFSA-GMO-NL-2010-78) for the placing on the market of herbicide-tolerant, increased oleic acid genetically modified soybean MON 87705 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

This scientific opinion is a risk assessment of the genetically modified, herbicide-tolerant, increased oleic acid soybean MON 87705 for food and feed uses, import and processing. MON 87705 contains the soybean *FAD2-1A/FATB1-A* gene fragments down-regulating endogenous FAD2 and FATB enzymes and the CP4 *epsps* gene cassette conferring tolerance to glyphosate-containing herbicides. Bioinformatic analyses and genetic stability studies did not raise safety issues. The levels of the CP4 EPSPS protein in soybean MON 87705 have been sufficiently analysed. MON 87705 differs from the conventional counterpart in the fatty acid profile (proportion of (C18:1) oleic acid increased and proportions of (C18:2) linoleic acid and (C16:0) palmitic acid decreased) in seeds and the presence of the CP4 EPSPS protein. Scientific risk assessment of soybean MON 87705 was carried out in the context of the intended use as specified by the applicant, namely its use for food and feed as any conventional soybean except for the oil derived from soybean MON 87705, which is to be used in margarine, salad dressing, mayonnaise and home-use liquid vegetable oil, excluding the use of soybean MON 87705 oil for commercial frying.

The safety assessment identified no concerns regarding potential toxicity and allergenicity of the CP4 EPSPS protein. The altered fatty acid profile did not raise concerns regarding toxicity. The overall allergenicity of the whole plant was not changed by the genetic modification. The estimated changes in intake levels of these fatty acids do not raise nutritional concerns in the context of the intended use as specified by the applicant. A feeding study on broiler chickens confirmed that defatted meal of soybean MON 87705 is as nutritious as meals produced from its conventional counterpart and non-GM reference varieties. There are no indications of an increased likelihood of establishment and spread of feral soybean plants. Considering its intended uses, environmental risks associated with an unlikely, but theoretically possible, horizontal gene transfer from soybean MON 87705 to bacteria have not been identified. Potential biotic and abiotic interactions of soybean MON 87705 were not considered to be an issue owing to the low level of exposure. The monitoring plan is in line with the intended uses of soybean MON 87705.

[1] On request from the Competent Authority of the Netherlands for an application (EFSA-GMO-NL-2010-78) submitted by Monsanto, Question No EFSA-Q-2010-00165, adopted on 28 September 2012.

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[3] Acknowledgement: The Panel wishes to thank the members of the Working Groups on Molecular Characterisation, Food and Feed and Environment for the preparatory work on this scientific opinion; Anke Meisner, Thomas Frenzel and Hildegard Przyrembel as external experts; and the EFSA's staff members Anna Christodoulidou, Zoltán Divéki and Christina Ehlert for the support provided to this scientific opinion.

Suggested citation: EFSA Panel on Genetically Modified Organisms (GMO); Scientific Opinion on application (EFSA-GMO-NL-2010-78) for the placing on the market of herbicide tolerant genetically modified soybean MON 87705 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal (2012);10(10):2909. [34 pp.] doi:10.2903/j.efsa.2012.2909. Available online: www.efsa.europa.eu/efsajournal

The EFSA GMO Panel considers that the information available for soybean MON 87705 addresses the scientific comments raised by the Member States and states that soybean MON 87705, as described in the application, is as safe as its conventional counterpart with respect to potential effects on human and animal health and the environment in the context of its intended uses as proposed by the applicant.



**KEY WORDS**

GMO, soybean (*Glycine max*), MON 87705, herbicide tolerant, increased oleic acid, RNAi, CP4 EPSPS, human and animal health, import and processing, Regulation (EC) No 1829/2003

## SUMMARY

Following the submission of an application (EFSA-GMO-NL-2010-78) under Regulation (EC) No 1829/2003 from Monsanto, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) was asked to deliver a scientific opinion on the safety of herbicide-tolerant, increased oleic acid genetically modified (GM) soybean MON 87705 (Unique Identifier MON-87705-6) for food and feed uses, import and processing.

In delivering its scientific opinion, the EFSA GMO Panel considered the application EFSA-GMO-NL-2010-78, additional information supplied by the applicant, scientific comments submitted by the Member States and relevant scientific publications. The scope of application EFSA-GMO-NL-2010-78 is for food and feed uses, import and processing of soybean MON 87705 within the European Union as any non-GM soybean but excludes cultivation in the EU. The EFSA GMO Panel evaluated soybean MON 87705 with reference to the intended uses defined by the applicant and appropriate principles described in its Guidance Document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed (EFSA, 2006a, 2011a). The applicant stated that the fatty acid properties of soybean MON 87705 oil would be suitable for replacement of liquid vegetable oils currently used in margarine, salad dressing, mayonnaise and spread, and home-use liquid oil. The applicant did not provide data which would allow a nutritional assessment of soybean MON 87705 oil when used for commercial frying (*i.e.* high-temperature and repeated frying). Therefore, the nutritional assessment of soybean MON 87705 oil performed by the GMO Panel in this Opinion excludes commercial frying.

The scientific risk assessment included molecular characterisation of the inserted DNA, evaluation of the levels of the CP4 EPSPS protein and the increased oleic acid phenotype. An evaluation of the comparative analyses of composition, agronomic and phenotypic traits was undertaken, and the safety of the new proteins and the whole food/feed was evaluated with respect to potential toxicity, allergenicity and nutritional wholesomeness. An evaluation of the environmental impacts and the post-market environmental monitoring plan was undertaken.

Soybean MON 87705 was transformed using *Agrobacterium tumefaciens* (renamed as *Rhizobium radiobacter*) and expresses the CP4 *epsps* gene from *Agrobacterium* sp. CP4 coding for 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS), which renders MON 87705 tolerant to glyphosate-containing herbicides. Soybean MON 87705 also expresses fragments of the endogenous *FAD2-1A* and *FATB1-A* genes resulting, through RNA interference, in the decreased levels of fatty acid Δ12-desaturase (FAD2) and palmitoyl acyl carrier protein thioesterase (FATB) enzymes, and in turn an increased oleic acid phenotype.

The molecular characterisation data establish that genetically modified soybean MON 87705 contains a single insert, consisting of the intact copies of the *FAD2-1A/FATB1-A* and CP4 *epsps* expression cassettes. No other parts of the plasmid used for transformation are present in the transformed plant. Results of the bioinformatic analysis of the 5′ and 3′ flanking sequences and ORFs within the insert and spanning the junction sites did not indicate a safety issue. The stability of the inserted DNA was confirmed over several generations and a Mendelian inheritance pattern was demonstrated.

The GMO Panel considered the total compositional, phenotypic and agronomic data supplied and the observed compositional differences between soybean MON 87705 and its conventional counterpart in the light of the field trial design, measured biological variation and the level of the studied compounds in soybean reference varieties, and concludes that soybean MON 87705 differs from the conventional counterpart and other non-GM soybean reference varieties only in the fatty acid profile and the newly expressed protein CP4 EPSPS, as intended.

No toxicity of the CP4 EPSPS protein was observed in an acute oral toxicity study in mice. The protein was rapidly degraded under simulated gastric conditions. In bioinformatics studies the protein showed no homology to known toxic proteins and allergens. A subchronic 90- day feeding study in

rats using diets including defatted meal derived from soybean MON 87705 provided no indications of adverse effects. Testing of extracts from soybean MON 87705 and the conventional counterpart A3525 with sera from patients allergic to soybeans showed that the allergenicity of the whole plant had not been changed due to the genetic modification. A 42-day feeding study in broiler chickens demonstrated that diets formulated with defatted meal from soybean MON 87705 are as nutritious as diets with defatted meal from the conventional counterpart and non-GM soybean reference varieties. The GMO Panel is of the opinion that soybean MON 87705 is as safe as its conventional counterpart and non-GM soybean reference varieties in the context of the intended uses as proposed by the applicant. The altered fatty acid profile did not raise concerns regarding toxicity.

The nutritional assessment is focused on the intended increase of oleic acid (C18:1) and the accompanying decreases of linoleic acid (C18:2) and palmitic acid (C16:0), of which the levels were outside the ranges of the natural variation. The EFSA GMO Panel concludes that the estimated changes in fatty acid intake resulting from the replacement of conventional vegetable oils with oil from soybean MON 87705 do not raise nutritional concerns in the context of the intended use, as specified by the applicant. The applicant did not provide data which would allow a nutritional assessment of soybean MON 87705 oil when used for commercial frying (*i.e.* high-temperature and repeated frying). The EFSA GMO Panel is of the opinion that a specific nutritional assessment is required in case oil derived from soybean MON 87705 is used for food applications which have not been considered in the intake assessment provided to the Panel, *e.g.* commercial frying.

The scope of application EFSA-GMO-NL-2010-78 is for food and feed uses, import and processing and does not include cultivation. Considering the intended uses of soybean MON 87705, the environmental risk assessment is concerned with the indirect exposure mainly through manure and faeces from animals fed seed produced by soybean MON 87705 and with the accidental release into the environment of viable seeds produced by soybean MON 87705 during transport and processing.

In case of accidental release into the environment of viable seeds of soybean MON 87705 during transportation and processing, there are no indications of an increased likelihood of establishment and spread of feral soybean MON 87705 plants, except in the presence of glyphosate-based herbicides. In addition, the low levels of environmental exposure of these GM soybean plants and the newly expressed protein through other routes indicate that the risk to non-target organisms is extremely low. The EFSA GMO Panel considers that it is unlikely that the recombinant DNA in soybean MON 87705 transfers to bacteria. A risk caused by a rare, but theoretically possible, transfer of the recombinant genes from soybean MON 87705 to bacteria in the environment has not been identified by the GMO Panel because expression of these genes would not provide any selective advantage in the context of its intended use. The scope of the post-market environmental monitoring plan provided by the applicant and the reporting intervals are in line with the intended uses of soybean MON 87705.

The EFSA GMO Panel considers that information available for soybean MON 87705 addresses the scientific comments raised by Member States and considers that the soybean MON 87705 assessed in this application is as safe as its conventional counterpart in the context of its intended uses as proposed by the applicant (*i.e.* use for food and feed as any conventional soybean except for the oil derived from soybean MON 87705, which is to be used in margarine, salad dressing, mayonnaise and home-use liquid vegetable oil, excluding the use of soybean MON 87705 oil for commercial frying).

The EFSA GMO Panel concludes that soybean MON 87705, as described in this application, is unlikely to have any adverse effect on human and animal health and the environment, in the context of its intended uses as proposed by the applicant.

Considering the altered composition and nutritional values of soybean MON 87705, 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 has proposed that, for example, operators handling products containing or consisting of oil produced from MON 87705 shall be required to label these products with the words “increased oleic acid oil produced from genetically

modified soybean". The GMO Panel notes that the compositional data summarised above (section 4.1.2) show that the fatty acid composition of seeds of soybean MON 87705 and derived oil has indeed been changed in relation to the conventional counterpart, including an increased content of oleic acid in MON 87705 beyond the range of non-GM reference varieties and literature values. The proposed labelling does not specifically mention the intended uses of soybean MON 87705 oil. The GMO Panel recommends adding the specific uses, *i.e.* "only for use in margarine, salad dressing, mayonnaise and spread, and for home-use".

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## BACKGROUND

On 25 February 2010, the European Food Safety Authority received from the Dutch Competent Authority an application (Reference EFSA-GMO-NL-2010-78) for authorisation of genetically modified (GM) soybean MON 87705 (Unique Identifier MON-87705-6) submitted by Monsanto within the framework of Regulation (EC) No 1829/2003 on GM food and feed.[4] After receiving the application EFSA-GMO-NL-2010-78 and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed the Member States and the European Commission, and made the summary of the application publicly available on the EFSA website. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of Regulation (EC) No 1829/2003. On 13 August 2010, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the European Commission and consulted nominated risk assessment bodies of the Member States, including the national Competent Authorities within the meaning of Directive 2001/18/EC[5], 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 19 November 2010) within which to make their opinion known.

The Scientific Panel on Genetically Modified Organisms of EFSA (EFSA GMO Panel) carried out a scientific risk assessment of the GM soybean MON 87705 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. The EFSA GMO Panel carried out the safety evaluation in accordance with the appropriate principles described in the applicable Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006a, 2011a). In addition, the scientific comments of the Member States, the additional information provided by the applicant, and relevant scientific publications were taken into consideration.

On 5 November 2010, 3 February 2011, 22 March 2011 and on 11 November 2011 the EFSA GMO Panel requested additional information from the applicant. The applicant provided the requested information on 17 January 2011, 25 March 2011, 21 June 2011 and on 29 December 2011.

In giving its opinion on soybean MON 87705 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 6 months was extended accordingly, in line with Articles 6(1), 6(2), 18(1), and 18(2) of Regulation (EC) No 1829/2003.

According to Regulation (EC) No 1829/2003, this scientific opinion is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation and thus will be part of the EFSA overall opinion in accordance with Articles 6(5) and 18(5).

## TERMS OF REFERENCE

The EFSA GMO Panel was requested to carry out a scientific assessment of soybean MON 87705 (Unique Identifier MON-87705-6) 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

[4] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. Official Journal of the European Communities, L268, 1–23.

[5] 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. Official Journal of the European Communities, L106, 1–38.

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 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. 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

Genetically modified soybean MON 87705 (Unique Identifier MON-87705-6) was evaluated with reference to its intended uses as proposed by the applicant (see below), taking account of the appropriate principles described in the applicable Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006a, 2011a). The scientific risk assessment presented here is based on the information provided in the application, as well as additional information from the applicant, scientific comments submitted by the Member States and relevant scientific publications.

Soybean MON 87705 has been modified to contain a increased oleic acid content and reduced linoleic acid content in seeds and tolerance of the whole plant to herbicides containing the active component glyphosate. The increased oleic acid phenotype is achieved by introducing fragments of the soybean *FAD2-1A* and *FATB1-A* genes, under the control of a promoter predominantly active in seeds. The genetic modification results in an inhibition of the expression of the *FAD2-1A* and *FATB1-A* genes by RNAi interference (RNAi), resulting in reduced levels of the corresponding fatty acid Δ12-desaturase and palmitoyl acyl carrier protein thioesterase enzymes. The transport of the saturated fatty acids out of the plastid is thus decreased, the conversion of oleic acid to linoleic acid is inhibited (linoleic acid decreases), and the oleic acid level increases. In addition, soybean MON 87705 expresses a 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. CP4 (CP4 EPSPS) in all cells of the plant, thereby conferring tolerance to glyphosate-containing herbicides.

The applicant stated that the fatty acid properties of soybean MON 87705 oil would be suitable for replacement of liquid vegetable oils currently used in margarine, salad dressing, mayonnaise and spread, and home-use liquid oil. The applicant did not provide data which would allow a nutritional assessment of soybean MON 87705 oil when used for commercial frying (*i.e.* high-temperature and repeated frying). Therefore, the nutritional assessment of soybean MON 87705 oil performed by the GMO Panel in this Opinion excludes commercial frying.

## 2. ISSUES RAISED BY MEMBER STATES

The issues raised by the Member States are addressed in Annex G of the EFSA overall opinion[6] and have been considered in this scientific opinion.

## 3. MOLECULAR CHARACTERISATION

### 3.1. Evaluation of relevant scientific data

#### 3.1.1. Transformation process and vector constructs[7]

Soybean MON 87705 was developed through *Agrobacterium*-mediated (also known as *Rhizobium radiobacter*) transformation of meristem tissue from the embryos of germinated seeds of the conventional soybean variety A3525. The regeneration did not include a callus phase. The plasmid vector PV-GMPQ/HT4404 contained two T-DNAs. T-DNA I contained:

1) a cassette intended to confer glyphosate tolerance by introducing a gene coding for the CP4 EPSPS enzyme, which has much reduced affinity for glyphosate. This cassette was constructed from promoter sequences from *Figwort mosaic virus* (enhancer of 35S RNA promoter) and *Arabidopsis thaliana* (from the *Tsf1* gene, which encodes elongation factor EF-1 alpha), a 5′ untranslated leader sequence (exon 1) and intron with flanking exon sequence from the *Tsf1* gene, a chloroplast-targeting sequence from *A. thaliana* (from the *ShkG* gene, which encodes EPSPS), a

[6] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2010-00165
[7] Technical dossier/Sections C and D1.

codon-optimised CP4 *epsps* coding sequence from *Agrobacterium* sp. CP4 (*aroA* gene, used as selectable marker for transformation) and a 3′ untranslated region from garden pea (from the *RbcS2* gene, which encodes RuBisCO small subunit 2).

2) a cassette intended to modify the fatty acid profile of soybean seeds by suppressing the expression of two endogenous genes in the fatty acid biosynthesis pathway. This cassette was constructed from a promoter and a leader sequence from soybean (the *Sphas1* gene, which encodes a seed storage protein that facilitates transcription in seeds and limits the modified oil composition to be expressed in seed tissue), a partial sequence from the first intron of the soybean *FAD2-1A* gene (in the sense orientation; the gene encodes a fatty acid Δ12-desaturase that desaturates $18{:}1^{\Delta 9}$ oleic acid to $18{:}2^{\Delta 9,12}$ linoleic acid; down-regulation of the enzyme increases the level of oleic acid and decreases the level of linoleic acid) and a partial sequence from the 5′ untranslated region and the chloroplast-targeting sequence of the soybean *FATB1-A* gene (in the sense orientation; the gene encodes palmitoyl acyl carrier protein thioesterase, which terminates the fatty acid biosynthesis cycle to 16:0 palmitic acid; down-regulation of the expression of these two genes increases the synthesis of 18-carbon fatty acids). No 3′ untranslated region was present in this cassette.

T-DNA II contained a partial suppression cassette with the *FAD2-1A* and *FATB1-A* fragments (see point 2 above) in the antisense orientation to facilitate, together with T-DNA I, the formation of double-stranded RNA and thus RNA interference-mediated suppression (silencing) of the genes. In addition, this second T-DNA contained a 3′ untranslated region from pima cotton (*Gossypium barbadense*) (from the *H6* gene, which encodes a protein involved in secondary cell wall assembly) for transcription termination.

The vector backbone contained elements derived from bacteria which are necessary for the maintenance and selection of the vector in bacteria: *aad*A (encodes an enzyme that confers spectinomycin and streptomycin resistance; for selection of plasmid in bacteria), *ori-pBR322* (origin of replication; for maintenance of plasmid in *Escherichia coli* during the construction of the cassettes), *rop* (encodes repressor of primer protein; for maintenance of plasmid copy number in *E. coli*), and *oriV* (origin of replication; for maintenance of plasmid in *Agrobacterium*).

### 3.1.2. Transgene constructs in the GM plant[8]

To determine the copy number of transgenic constructs inserted and the insertion site(s), and to confirm the absence of plasmid backbone sequences in soybean MON 87705, molecular characterisation of the soybean was conducted by Southern analysis. Leaf samples from soybean MON 87705 generation $R_3$, which had been obtained through three rounds of self-pollination of the original transformed event in the A3525 soybean genetic background ($R_0$) and were homozygous for the T-DNA insert, were used in the analyses. The non-transformed soybean line A3525 was used as a conventional counterpart.

The molecular approaches used were acceptable in terms of both coverage and sensitivity. The 10 overlapping probes covered the whole plasmid vector. The analyses showed the presence of a single copy of T-DNA I- and T-DNA II-derived sequences that were integrated into a single locus in soybean MON 87705. The absence of all elements from the plasmid backbone (*e.g.* the *aad*A resistance gene) was properly demonstrated.

Sequence analysis and PCR were used to determine the organisation of genetic elements within the MON 87705 insert and for the comparison of the insertion site with the parental soybean line A3525. The insert contains a complete CP4 *epsps* cassette. Furthermore, T-DNA I and T-DNA II are integrated adjacent to each other in a way that a complete *FAD2-1A/FATB1-A* suppression cassette is formed which expresses RNA containing an inverted repeat of the *FAD2-1A* and *FATB1-A* gene

[8] Technical dossier/Section D2.

fragments. The novel junction between the two T-DNAs is somewhat modified, including ca. 10 % truncation (30 bp) of the *FATB1-A* fragment in T-DNA II. Comparison of the insertion site in soybean MON 87705 with the parental line indicated a 36 bp deletion. In addition, a 3′ flanking fragment of 2374 bp was duplicated and is present at the 5′ end of the insertion as a nearly perfect direct repeat.

Bioinformatic analysis of the pre-insertion locus did not indicate the disruption of known endogenous soybean genes. 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 to appropriate databases for similarities to known allergens and toxins by using suitable algorithms. None of the putative ORF amino acid sequences identified at the junctions and in the MON 87705 insert showed biologically significant sequence similarities with known toxins or allergens. These bioinformatic analyses support the conclusion that, even in the unlikely event that any of the new ORFs at the junctions were translated, they would not raise a safety issue.

### 3.1.3. Information on the expression of the insert[9]

The sense and antisense segments of *FAD2-1A* and *FATB1-A* integrated into the soybean genome express RNA that contains an inverted repeat of the gene segments. This transcript produces double-stranded RNA that via RNA interference leads to the degradation of endogenous FAD2-1A and FATB1-A mRNAs. This was verified by Northern analyses with probes hybridising to the coding regions and 3′ UTRs of the mRNAs, which indicated large decreases in FAD2-1A and FATB1-A mRNA levels in soybean MON 87705 seeds. These decreases were also reflected in the fatty acid profile of the seeds, *i.e.* decreased level of saturated fatty acids (palmitic and stearic acids), increased level of mono-unsaturated oleic acid and decreased level of polyunsaturated linoleic acid compared with conventional soybean.

The only newly expressed protein in soybean MON 87705 is CP4 EPSPS. The levels of the protein were analysed by a validated ELISA system from $R_5$ and $R_6$ generations developed by self-pollination from the original transformant $R_0$. Samples were collected from leaf, seed, forage and root tissues of soybean MON 87705 treated with glyphosate and harvested from five field sites in Chile (2007/2008 growing season) and four field sites in the USA (2008 growing season). Considering the scope of the application, protein levels in seed and forage are considered most relevant, and are summarised in Table 1.

[9] Technical dossier/Section D3

**Table 1:** Means and ranges of CP4 EPSPS levels in soybean MON 87705 (µg/g dw)

| | | Chile 2007–2008 | USA 2008 |
|---|---|---|---|
| seed | mean | 110 | 160 |
| | range | 40–210 | 130–190 |
| forage | mean | 120 | 160 |
| | range | 77–160 | 94–220 |

### 3.1.4. Inheritance and stability of the inserted DNA[10]

The inserted DNA in soybean MON 87705 is integrated in the nuclear genome. Genetic stability was evaluated by studying the inheritance and segregation pattern of the introduced genetic material in four generations. Southern analysis using one restriction enzyme and two probes spanning a portion of the T-DNA I and T-DNA II and covering both border regions was carried out from plants of the $R_3$ to $R_6$ generations. The analysis did not indicate any unexpected bands that would indicate instability of the insert. At generations $R_2$ to $R_4$ the fixed homozygous plants were tested for the segregation pattern for the H6 3′ untranslated region in the insert. Furthermore, the inheritance and stability was assessed from plants of the $F_2$ to $F_5$ generations produced after $R_4$ plants were crossed with another soybean variety to produce hemizygous seed and then self-pollinating. Altogether ca. 4 700 plants were tested.

The $F_3$ generation gave inconclusive results, perhaps because of a small sample size (81 tested plants). The sample size was subsequently increased. The results from generations $F_2$, $F_4$ and $F_5$ indicate that the inserted DNA segregates following a typical pattern of Mendelian inheritance expected for a single genetic locus.

## 3.2. Conclusion

Molecular characterisation data establish that soybean MON 87705 contains a single insert. Bioinformatic analyses of the ORFs spanning the junction sites within the insert or between the insert and genomic DNA did not indicate hazards. Levels of the CP4 EPSPS protein have been sufficiently analysed in soybean MON 87705 and the stability of the inserted DNA was confirmed over several generations. The EFSA GMO Panel concludes that the molecular characterisation does not raise safety issues.

# 4. COMPARATIVE ANALYSIS

## 4.1. Evaluation of relevant scientific data

### 4.1.1. Choice of comparator and production of material for the compositional assessment[11]

In the compositional, phenotypic and agronomic studies, the soybean MON 87705 was compared with the conventional counterpart A3525, which is a commercial soybean variety with a similar genetic background to soybean MON 87705. Soybean MON 87705 and its conventional counterpart were grown under the same agronomic conditions (apart from treatments of MON 87705 with glyphosate herbicides) in replicated plots. The field trials for comparative compositional analyses were carried out in Chile at five different geographical sites in the season 2007/2008 and in the USA at five different geographical sites in the season 2008. One site in the USA was excluded from the analysis because of technical problems (control plots were mistakenly treated with glyphosate and did not survive). The applicant included a total of 19 non-GM soybean reference varieties in Chile in 2007/2008 and 18

[10] Technical dossier/Section D5.
[11] Technical dossier/Section D7.2.

non-GM soybean reference varieties in the USA in 2008. In the 2008 growing season plots where soybean MON 87705 was not treated with glyphosate herbicides were also included.

Data obtained for soybean MON 87705 and its conventional counterpart for each studied parameter regarding phenotypic, agronomic and compositional characteristics were compared with the ranges of these parameters defined by the non-GM reference soybean varieties. Data to define natural ranges were also derived from the literature, including those available in the ILSI Crop Composition Database (ILSI-CCD, 2006).[12]

### 4.1.2. Compositional analysis[13]

Soybean seeds were assessed by proximate analysis and for specific fibre fractions, as well as for 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 by proximate analysis and for specific fibre fractions. The selection of compounds followed the recommendations by OECD (2000). The data on each analyte were statistically analysed for potential differences in their levels in soybean MON 87705 and the conventional counterpart within-site and across-sites (data from all sites combined). Statistically significantly different values ($P < 0.05$) were compared with the range of values observed in the reference soybean varieties, as well as in the literature.

Consistent statistically significant compositional differences between soybean MON 87705 and its conventional counterpart were found for the fatty acid profile, demonstrating the intended effect of the genetic modification.

The most prominent changes compared with the non-GM comparator, consistently statistically significant, were an increase in the content of oleic acid (C18:1) and decrease in linoleic acid (C18:2) and palmitic acid (C16:0). The analysed differences fall also outside the range in content of these fatty acids in the reference varieties and those reported in the literature.

Smaller although significant changes were observed for a decrease in stearic acid (C18:0, outside the range in the soybean reference varieties for the trials in Chile and inside the range of reference varieties for the trials in the USA and those reported in the literature), linolenic acid (C18:3, outside the range in the soybean reference varieties for the trials in the USA and inside the range of reference varieties for the trials in Chile and those reported in the literature), arachidic acid (C20:0, within the range of in the reference soybean varieties as well as within the range of literature data) and behenic acid (C22:0; only in the US trials) and an increase in eicosenoic acid (C20:1, outside the range of the soybean reference varieties, but within the range described in the literature). The data are summarised in Table 2. Elaidic acid (C18:1 9t) in seeds of MON 87705 was consistently found to be below the assay limit of quantification (0.020 %).

[12] ILSI-CCD; International Life Sciences Institute-Crop Composition Database, http://www.cropcomposition.org
[13] Technical dossier/Section D7.1.

**Table 2:** Mean fatty acid contents (% of total fatty acids) of seeds of soybean MON 87705, its conventional counterpart and the reference varieties harvested from field trials in Chile (2007/2008) and the USA (2008). The differences are statistically significant ($P < 0.05$ in the combined-site analysis) unless indicated otherwise

| Fatty acid | Chile 2007/2008 | | | USA 2008 | | | | Literature range[a] |
|---|---|---|---|---|---|---|---|---|
| | MON 87705, glyphosate-treated | A3525 | Range in non-GM reference varieties | MON 87705, glyphosate-treated | MON 87705, untreated | A3525 | Range in non-GM reference varieties | |
| Palmitic C16:0 | 2.36 | 10.38 | 8.78–11.51 | 2.52 | 2.52 | 11.68 | 9.15–11.64 | 9.55–15.77 |
| Stearic C18:0 | 3.31 | 4.50 | 3.82–7.21 | 3.11 | 3.14 | 4.21 | 3.10–4.58 | 2.70–5.88 |
| Oleic C18:1 | 76.47 | 22.81 | 20.77–27.19 | 69.04 | 69.34 | 20.84 | 19.22–25.77 | 14.3–32.2 |
| Linoleic C18:2 | 10.10 | 52.86 | 48.62–54.74 | 16.04 | 15.84 | 53.30 | 50.01–55.35 | 42.3–58.8 |
| α-linolenic C18:3 | 6.69 | 8.02 | 5.89–9.11 | 8.51 | 8.37 | 9.22 | 7.22–10.68 | 3.00–12.52 |
| Arachidic C20:0 | 0.30 | 0.34 | 0.28–0.54 | 0.25 | 0.26 | 0.30 | 0.23–0.35 | 0.163–0.482 |
| Eicosenoic C20:1 | 0.34 | 0.19 | 0.15–0.22 | 0.26 | 0.26 | 0.15 | 0.15–0.21 | 0.140–0.350 |
| Behenic C22:0 | 0.29* | 0.30* | 0.29–0.46 | 0.26 | 0.27 | 0.30 | 0.29–0.39 | 0.277–0.595 |

[a]The literature data include data from the ILSI crop composition database (2006).
*Not statistically significant in these field trials.

For the other key constituents, recommended by OECD, including anti-nutrients and other secondary metabolites (isoflavones), no consistent alteration was found between soybean MON 87705 and the conventional counterpart within sites and across sites. Inconsistent statistically significant differences were generally small and the measured values fell within the range in the level of the various constituents in the commercial reference soybean varieties or literature data. Therefore, these differences were not considered to be biologically relevant by the EFSA GMO Panel.

Regarding forage, statistically significant differences in the proximate and fibre levels were few, small and not consistently observed. The values fell within the range of the reference soybean varieties and that described in literature data.

The GMO Panel considered the total compositional data supplied and the observed compositional differences between soybean MON 87705 and its conventional counterpart in the light of the field trial design, measured biological variation and the level of the studied compounds in non-GM soybean reference varieties, and concludes that no biologically relevant differences were identified between soybean MON 87705 and the conventional counterpart and other non-GM soybean reference varieties, except for the fatty acid profile and the newly expressed protein CP4 EPSPS.

### 4.1.3. Agronomic traits and GM phenotype[14]

The applicant provided information on agronomic performance, phenotypic characteristics and ecological interactions of soybean MON 87705 and its conventional counterpart from field trials performed in the USA in 2007 (17 field trial sites) and in 2008 (four field trial sites). The applicant included 13 non-GM reference varieties in 2007 and 10 non-GM reference varieties in 2008. The characteristics evaluated were early stand count, final stand count, seedling vigour, days to 50 % flowering, plant height, lodging, pod shattering, seed moisture, 100-seed weight, test weight, yield, growth stage, flower colour, plant pubescence, abiotic stress response, disease damage, arthropod damage, arthropod pest and beneficial arthropod abundance. When analysed across locations, statistically significant differences were observed for some agronomic parameters, *i.e.* in 2007 for early and final stand count, flowering and 100-seed weight, and in 2008 for 100-seed weight. However, when analysed by site, statistically significant differences were not consistently observed.

Ecological interactions were assessed qualitatively at a total of 21 sites and arthropod abundance data were collected quantitatively from eight sites. Differences between MON 87705 and its conventional control in disease and arthropod damage were not consistently observed and the severity of damage in the MON 87705 plots was within the range observed in conventional reference varieties (2007 growing season). In 2008 no such differences were observed. As for arthropod abundance, statistically significant differences between MON 87705 and the conventional counterpart were not consistently observed and fell within the range observed in conventional reference varieties, with the exception of two values at a single site in growing season 2008, where the mean abundance values of two insect species were higher for MON 87705.

As the magnitudes of the differences in agronomic parameters were small, and they fell within the ranges observed for commercial soybeans, the GMO Panel found these differences to be of no biological relevance.

## 4.2. Conclusion

The GMO Panel considered the total compositional, phenotypic and agronomic data supplied and the observed compositional differences between soybean MON 87705 and its conventional counterpart in the light of the field trial design, measured biological variation and the level of the studied compounds in soybean reference varieties, and concludes that soybean MON 87705 differs from the conventional counterpart and other non-GM soybean reference varieties only in the fatty acid profile and the newly expressed protein CP4 EPSPS, as intended.

# 5. FOOD/FEED SAFETY ASSESSMENT

## 5.1. Evaluation of relevant scientific data

### 5.1.1. Product description and intended use[15]

The scope of application EFSA-GMO-NL-2010-78 is for food and feed uses, import and processing of soybean MON 87705. The main product for human use is soybean oil. In addition, soybean is used for the production of soybean milk, protein concentrates, flour, sprouts, baked or roasted soybeans, tofu, soybean sauce and other products for human consumption. Defatted soybean meal is used as a source of protein in animal feed, sometimes in combination with soybean hulls. There is also a limited direct use of full-fat soybeans as animal feed.

Soybean MON 87705 and all food, feed and processed products derived from soybean MON 87705 are expected to replace a portion of similar products from commercial soybean. Oil from this soybean might also replace oils from other sources than soybean. The applicant stated that soybean MON 87705 oil is targeted for applications such as margarine, salad dressing, mayonnaise and spread,

[14] Technical dossier/Section D7.4.

and home-use liquid vegetable oil, but not for commercial frying (*i.e.* high-temperature and repeated frying).

### 5.1.2. Effects of processing[16]

Soybean MON 87705 will undergo existing methods of production and processing used for commercial soybean. No novel method of production and processing is envisaged.

Considering the compositional differences observed for the raw agricultural commodity, a detailed comparative compositional analysis of processed products derived from soybean MON 87705 was carried out: the untreated soybean MON 87705, the conventional counterpart and non-GM reference varieties were included in that study. The seeds were processed into refined bleached deodorised (RBD) oil, isolated soy protein, toasted defatted meal and crude lecithin. RBD oils were analysed for fatty acid composition and vitamin E. Toasted defatted meal was assessed by proximate analysis, and for fibre fractions, amino acids and two anti-nutrients (phytic acid and trypsin inhibitor). Isolated soy protein was analysed for amino acids. Crude lecithin was analysed for phosphatides. Seed samples to prepare soybean processed fractions were collected from field trials where MON 87705 and the conventional counterpart A3525 were grown in replicated plots at two sites in the USA during the 2007 growing season. Additionally 12 non-GM reference varieties were used for the analysis.

The intended effects of the genetic modification and the effects on the fatty acid pattern already seen in the analysis of unprocessed soybean seeds were also reflected in the composition of RBD oil obtained from MON 87705. Additional differences were small and fell within the ranges of these constituents defined by levels in non-GM soybean reference varieties or the literature data with the exception of heptadecenoic acid (C17:1 9c) and octadecadienoic acid (C18:2 6c, 9c) which are both minor fatty acids in soybean oil. The content of elaidic acid (C18:1 9t) analysed in RBD oil of soybean MON 87705 was low and not consistently above the assay limit of quantification.

Statistically significant differences in the other processed products were few and small. The values fell within the range of the non-GM reference soybean varieties and the range described in literature data.

### 5.1.3. Toxicology

#### 5.1.3.1. Protein used for safety assessment[17]

Given the low levels of the protein CP4 EPSPS expressed in soybean MON 87705, CP4 EPSPS protein produced in a recombinant *E. coli* strain was used in the safety assessment.

The C4 EPSPS protein produced in the leaf tissue of MON 87705 was subjected to N-terminal sequence analysis, MALDI-TOF MS analysis and SDS-PAGE electrophoresis. The equivalence between the plant produced and the previously characterized *E. coli*-derived C4 EPSPS protein was established by Western blot analysis, analysis of the enzymatic activity and glycosylation analysis.

Based on the identified similarity in structure and equivalence in physico-chemical properties and function between these proteins, the EFSA GMO Panel accepts the use of CP4 EPSPS derived from *E. coli* as appropriate substitute test material for the CP4 EPSPS protein present in soybean MON 87705 in the safety studies.

#### 5.1.3.2. Toxicological assessment of the expressed novel protein in soybean MON 87705[18]

The EFSA GMO Panel has previously evaluated the safety of the newly expressed CP4 EPSPS protein in single events (EFSA, 2008, 2011a), and no new information has appeared that requires the EFSA

[16] Technical dossier/Section D7.1.4.
[17] Technical dossier/Section D7.8.1.i.
[18] Technical dossier/Section D7.8.1.ii.

GMO Panel to alter its opinion that no safety concern is identified for humans and animals exposed to the protein. The applicant has provided data on the acute toxicity of the microbially produced CP4 EPSPS protein. The EFSA GMO Panel is of the opinion that the single dose acute oral toxicity study does not add relevant information for the safety assessment of this protein. Moreover results of *in vitro* degradation studies in pepsin-containing simulated gastric fluid and of a bioinformatic search for similarities to known toxins were provided. No protein was detectable after 15 s in the *in vitro* digestion studies, and the bioinformatics study indicated no similarity of the CP4 EPSPS protein to known toxins.

#### 5.1.3.3. Toxicological assessment of changed levels in natural constituents[19]

The compositional analysis of soybean MON 87705 confirmed changes in the fatty acid composition of the seeds. Based on the nature of the changes in the fatty acid profile, no concerns were raised regarding toxicity.

#### 5.1.3.4. Toxicological assessment of the whole GM food/feed[20]

The applicant has provided a subchronic (90 days) rat feeding study. Five groups, each consisting of 12 male and 12 female Sprague–Dawley rats, were fed diets containing 30 % defatted soybean meal. One group received defatted meal from soybean MON 87705, another group received defatted meal from the conventional counterpart A3525, whereas three additional groups received defatted meal from the reference soybean varieties Anand, UA4805 and Ozark. The animals were followed for mortality and moribundity as well as for clinical signs throughout the study. On the day of the necropsy, analyses were performed of haematology, serum chemistry and urine. Gross pathology, organ weight determinations and histological analyses of selected organs and tissues were performed. The data on body weight, cumulative body weight change, food consumption, clinical pathology and organ weight were compared between rats supplied a diet with defatted soybean meal from MON 87705 and A3525 (the conventional counterpart), and statistically significant differences were further assessed against the values obtained with the reference varieties and/or historical control data.

Throughout the treatment period there was no mortality and no unusual clinical observations were reported. Food consumption, body weight and cumulative body weight changes were comparable in all groups, and there were no statistically significant differences between the groups fed with MON 87705 and those fed with the conventional counterpart. Statistically significant differences from the control group observed in MON 87705-treated animals included lower absolute and percentage reticulocyte values in females and lower serum phosphorus concentration in males. The mean values for these parameters fell within the ranges of the respective mean values of the historical controls (*i.e.* animals fed diets with 30 % defatted meal from non-GM soybeans in previous 90-day studies). In the absence of changes in related parameters, these differences are regarded as incidental findings. A lower alanine aminotransferase activity in females was not regarded as an indication of toxicity. Organ weight determinations showed lower mean kidney weight relative to body weight in males. The mean value was within the range of the three conventional soybean varieties as well as within the historical control means, whilst the mean value for the control group fell outside these ranges. Thus, this finding results from a relatively high mean value of the control group. In females a higher mean adrenal weight relative to body weight was observed, which was still within the ranges of the historical control means. Females also showed a lower absolute but not relative heart weight as well as a lower thyroid/parathyroid weight (absolute and relative to brain weight). No differences in thyroid/parathyroid weight in relation to body weight were identified. Since, in addition, the macroscopic and histopathological examinations of the kidney, adrenal, heart and thyroid/parathyroid did not reveal findings which are attributable to soybean MON 87705, the observed differences are not considered toxicologically relevant. Microscopic examinations of other organs and tissues also showed no relevant differences in the incidence and severity of findings between the groups fed with soybean

[19] Technical dossier/Section D7.8.3.
[20] Technical dossier/Section D7.8.4.

MON 87705, the conventional counterpart as well as the three conventional varieties. The EFSA GMO Panel concludes that there are no indications of adverse effects after administration of diets containing defatted meal from soybean MON 87705 in this study.

### 5.1.4. Allergenicity

The strategies used when assessing the potential allergenic risk focus on the characterisation of the source of the recombinant protein(s), the potential of the newly expressed protein(s) 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 food. A weight-of-evidence approach is recommended, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (Codex Alimentarius, 2009; EFSA, 2006a; 2010a, 2011a).

#### 5.1.4.1. Assessment of allergenicity of the newly expressed proteins[21]

The gene encoding the CP4 EPSPS protein originates from *Agrobacterium* sp. CP4, which is an organism not considered allergenic (EFSA, 2008, 2011c).

Bioinformatics-supported comparisons of the amino acid sequence of the CP4 EPSPS with the sequences of known allergens were performed. These analyses included both an overall search for sequence alignments using the FASTA algorithm and a search for short identical stretches of at least eight contiguous amino acids. No similarity applying a criterion of 35 % identity over a window of 80 amino acids was identified and no identical stretches of at least eight contiguous amino acids were detected.

The studies on degradation of the CP4 EPSPS protein in simulated gastric fluid, which are also relevant for the assessment of potential allergenicity, were described in section 5.1.3.2. The results of these studies on *in vitro* degradation raised no concern.

Based on this information the EFSA GMO Panel considers that the protein CP4 EPSPS present in soybean MON 87705 is unlikely to be allergenic at the intended conditions of use of soybean MON 87705.

#### 5.1.4.2. Assessment of allergenicity of the whole GM plant[22]

Allergenicity of the whole plant could be increased as an unintended effect of the random insertion of the transgene in the genome of the recipient, for example through qualitative or quantitative modifications of the pattern of expression of endogenous proteins.

Because the soybean is a recognised allergenic food, the applicant has performed *in vitro* allergenicity studies with extracts of seeds from soybean MON 87705, its conventional counterpart and 17 different non-GM soybean reference varieties. The IgE binding of soybean proteins to sera from 13 individuals clinically documented allergic to soybean and five non-allergic individuals was quantified with an ELISA method in order to demonstrate that the allergenicity potential of soybean MON 87705 is not altered in comparison to conventional soybean varieties. The data obtained showed that the IgE binding was very similar between soybean MON 87705 and A3525 extracts with each of the sera tested with the exception of two sera, *i.e.* ME2 and MS07.

Additional data were obtained from 2-D Western blot analysis of human IgE binding to soybean MON 87705 and A3525 extracts using sera from eight allergic subjects, selected on a basis of high amounts of circulating IgE. The Western blot patterns obtained with both varieties were generally identical indicating no major differences in the number and migration properties of the IgE-reacting

[21] Technical dossier/Section D7.9.1.
[22] Technical dossier/Section D7.9.2.

proteins or in the intensities of the binding. Although some variation and differences in the patterns could be seen, these were minor and were not consistently observed for any specific soybean variety tested. In addition, the Western blot analysis did not show differences between soybean MON 87705 and A3525 extracts with the two sera, *i.e.* ME2 and MS07, for which some differences were observed in the ELISA IgE binding test. Upon request of the EFSA GMO Panel, the applicant tentatively identified certain putative soybean allergens on the basis of their migration in 2D gels (β-conglycinin subunits, glycinin subunits, P34 and glycinin subunit precursors, Kuniz trypsin inhibitor, agglutinin, Gly m4 and agglutinin fragment). There were no differences regarding these spots between soybean MON 87705 and its conventional counterpart.

Therefore, the EFSA GMO Panel is of the opinion that these studies do not indicate a modification of the overall allergenicity of soybean MON 87705 as compared with that of its conventional counterpart.

### 5.1.5. Nutritional assessment of GM food/feed

#### 5.1.5.1. Food

The nutritional assessment is focused on the intended increase of oleic acid (C18:1) and the accompanying decreases of linoleic acid (C18:2) and palmitic acid (C16:0). Changes in the levels of other fatty acids including the decrease of stearic acid (C18:0) and α-linolenic acid (C18:3) in MON 87705 compared with the conventional counterpart were small and the levels remained within the range of the fatty acid levels in the commercial reference varieties and described in the literature (see Table 2).

The slightly higher levels of the two minor fatty acids heptadecenoic acid (C17:1 9c) and octadecadienoic acid (C18:2 6c, 9c) in RBD oil from soybean MON 87705 (see section 5.1.2) do not raise concerns due to their low quantities and because they will be subject to normal fatty acid metabolic degradation.

*Intake information/exposure assessment*[23]

According to the applicant, soybean MON 87705 oil is intended for margarine, salad dressing, mayonnaise and spread, and home-use liquid soybean oil. This assessment excludes the use of soybean MON 87705 oil for commercial frying (see section 1).

Based on the intake data from UK as reported by Hulshof et al. (1999) and obtained from the United Kingdom's National Diet and Nutrition Survey (Henderson et al., 2003), the mean per capita intake of soybean oil from the target foods is estimated to be 2.7 g/day for adult men and 1.8 g/day for adult females. Accordingly, the applicant has estimated the effects on the daily fatty acid intake of replacing conventional soybean oil in the target foods by oil from soybean MON 87705 (Table 3).

[23] Technical dossier/Section D7.10.1 and Additional information, 29/12/2011.

**Table 3:** The expected changes of the daily intake of fatty acids as a result of substitution of soybean oil by MON 87705 derived oil in target foods.

| **Fatty acid** | **Expected effect on daily intake (g/day)** | | | | | |
|---|---|---|---|---|---|---|
| | **per capita** | | **per user** | | | |
| | | | **90th percentile** | | **97.5th percentile** | |
| | from | to | from | to | from | to |
| Palmitic acid (C16:0) | 0.2 | 0.1 | 0.6 | 0.1 | 0.9 | 0.2 |
| Stearic acid (C18:0) | 0.1 | 0.1 | 0.2 | 0.2 | 0.3 | 0.3 |
| Oleic acid (C18:1) | 0.5 | 1.7 | 1.1 | 4.0 | 1.8 | 6.6 |
| Linoleic acid (C18:2) | 1.2 | 0.2 | 2.7 | 0.5 | 4.4 | 0.9 |
| α-linolenic acid (C18:3) | 0.2 | 0.2 | 0.4 | 0.3 | 0.6 | 0.6 |

Because the contribution of soybean oil in the target products in this scenario is estimated to represent only 3 % of the daily total dietary fat intake, the substitution of soybean MON 87705 oil is expected to have only modest effects on the total fatty acid intake. Despite the anticipated slight decrease in the total polyunsaturated fatty acids (PUFAs), the daily PUFA intake would still be at 6.3 E% for males and 6.2 E% for females, which remains within the recommended range for the reduction of diet-related chronic diseases (6–10 E%, BNF 2004; WHO 2003).

The Panel notes that this replacement scenario is of limited informative value, because (1) it is restricted to replacement of soybean oil only in the four target foods and (2) it is based on intake data from the UK only, which may not be representative for other European countries.

The EFSA GMO panel asked the applicant to elaborate the assessment by taking into account the possibility that soybean MON 87705 oil will also replace vegetable oils other than soybean oil in food products. According to the response of the applicant soybean MON 87705 oil is regarded "not optimal" for commercial frying because of its PUFA content, and therefore the applicant did not consider this use. Regarding the eventual replacement of other vegetable oils by soybean MON 87705 oil, the applicant made new estimates on the basis of three scenarios:

- Scenario 1: 100 % substitution of soybean, canola and sunflower oils in the target foods
- Scenario 2: 100 % substitution of soybean oil, 50 % substitution of canola and sunflower oils, and
- Scenario 3: 50 % substitution of soybean, canola and sunflower oils.

The nutrient composition and food intake data used in the analysis were based on the UK National Diet & Nutrition Survey for adults aged 19 to 64 years (Henderson et al., 2003), the Food Standards Agency risk recipes database (FSA, 2002) and data on food disappearance from FAOSTAT Annual food consumption for the UK (FAOSTAT, 2011).

The mean daily per capita consumption of soybean, canola and sunflower oils from the target foods is estimated to be 8.9 g per day, corresponding to approximately 12 % of the total daily dietary fat intake of the UK adult population (10.8 g per day for males and 7.2 g per day for females). The corresponding figures for the 97.5th percentile are 33.2 g per day (40 g per day for males and 24.5 g per day for females; 27 % and 22 % of the total dietary fat).

The predicted percentage changes of the total dietary intake of relevant fatty acids, calculated on the basis of the daily consumption of target foods in scenario 1, are given in Table 4.

**Table 4:** The predicted percentage changes (% E) of dietary intake of fatty acids as a result of the total replacement (scenario 1) of other vegetable oils by MON 87705 oil in target foods.

| **Fatty acid** | **Average** | | **97.5th percentile** | |
|---|---|---|---|---|
| | **Adult males** | **Adult females** | **Adult males** | **Adult females** |
| Palmitic acid (C16:0) | –7.6 % | –7.6 % | –17.2 % | –14.0 % |
| Stearic acid (C18:0) | +3.2 % | +3.2 % | +7.2 % | +5.9 % |
| Oleic acid (C18:1) | +10.5 % | +10.5 % | +23.6 % | +19.2 % |
| Linoleic acid (C18:2) | –8.6 % | –8.6 % | –19.3 % | –15.7 % |
| α-linolenic acid (C18:3) | –1.6 % | –1.6 % | –3.5 % | –2.9 % |

The predicted increase in the intake of stearic acid in this scenario is due to the relatively increased C18:0 content (3.8 %) of soybean oil compared with the replaced canola oil (1.5 %). However, there would be a net decrease in the saturated fatty acid intake. There would be a substantial increase in oleic acid intake, while the PUFA intake would be markedly reduced. Nonetheless, according to the calculations for scenario 1 in the UK population supplied by the applicant, the levels of PUFA intake, even in the 97.5th percentile, would remain within the range of dietary recommendations for both n-3 PUFA and n-6 PUFA (1–2 % and 2.5–9 % of energy intake). In the other scenarios the changes in the PUFA intake are even more modest.

The Panel notes that the three scenarios are based on estimates of vegetable oil use in target foods in the UK only. The Panel notes that vegetable oil consumption varies considerably between European countries (average in some southern European countries approximately 30 g/day, 97.5th percentile 40–65 g/day). However, the changes in the exposure to different fatty acids due to partial or total replacement of the vegetable oils by MON 87705 oil would be to a certain extent moderated, since the fatty acid composition of MON 87705 oil is very similar to that of olive oil, which is the main vegetable oil consumed in these countries.

*Conclusion*

The main product for human use is the soybean oil, on which this assessment is focused. Since other possible food uses (*e.g.* soybean milk, tofu, direct consumption of seeds either as such or processed, etc.) are not expected to contribute significantly to the fatty acid intake and because there are no relevant compositional differences in the constituents of MON 87705 soybeans other than the modified fatty acids content, no nutritional consequences are expected.

The total replacement of commercial soybean, canola and sunflower oils in margarine, salad dressing, mayonnaise and spread, and home use of oils by oil derived from soybean MON 87705 would increase oleic acid (18:1) intake and decrease the intake of palmitic acid (C16:0). Both of these changes are in line with the current dietary recommendations (EFSA, 2010b) and do not pose a safety concern. The levels of the two minor fatty acids heptadecenoic acid (C17:1 9c) and octadecadienoic acid (C18:2 6c, 9c) in RBD oil from soybean MON 87705 (see section 5.1.2) do not raise concerns due to their low quantities and because they will be subject to normal fatty acid metabolic degradation. The intake levels of α-linolenic acid (C18:3) would be only modestly affected, while the anticipated decrease in the intake of cis n-6 polyunsaturated fatty acids (predominantly of linoleic acid (C18:2)) in both males and females is more pronounced. The EFSA GMO Panel concludes that the estimated changes in intake levels of these fatty acids resulting from replacement of conventional oil with oil

from soybean MON 87705 do not raise nutritional concerns in the context of the intended use, as specified by the applicant. Furthermore, the Panel is aware that the replacement model represents a theoretical extreme case and may overestimate the actual exposure.

The applicant did not provide data which would allow a nutritional assessment of soybean MON 87705 oil when used for commercial frying (*i.e.* high-temperature and repeated frying).

#### 5.1.5.2. Feed[24]

A 42-day feeding study on broiler chickens (800 broilers) was performed according to ILSI (2003) recommendations. Groups consisting of 50 male and 50 female birds (five pens per sex) per group were fed diets containing defatted meal from either soybean MON 87705, the conventional counterpart (A3525) or any of six non-GM reference varieties.[25] The inclusion rate of defatted soybean meal in the starter diet was approximately 33 % and in the grower/finisher diet around 30 %. Soybean oil was added up to approximately 3 % into all diets. The diets were adjusted for their contents in protein, specific amino acids and minerals.

The average mortality was low in the study, 1.1 % across all the dietary treatments during the first 7 days and 1.3 % during the remaining period.

No treatment-related statistically significant differences in performance parameters such as weight gain or feed-to-gain ratio were observed between the soybean MON 87705-fed group and the control group. The final weights of animals at the end of the experiment were 2.61 kg and 2.62 kg in these groups, respectively, while the feed-to-gain ratio was 1.61 in both groups. The ranges of these parameters in the groups treated with non-GM reference varieties were 2.6–2.7 kg and 1.60–1.65 kg. There were also no effects on carcass yield or meat quality.

The use of soybean oil in animal feed is limited. Soybean oil may in some cases be added in small amounts (0.5–3 %) to mixed feed (especially for poultry and pigs) in order to avoid dust, to improve the quality/stability of pellets and to add energy to the diets. The modification of the fatty acid pattern in the oil (more oleic acid (C18:1); less linoleic acid (C18:2)) increases the oxidative stability of the oil and no negative effects on feeds are to be expected.

### 5.1.6. Post-market monitoring of GM food/feed

The scientific risk assessment concluded that no data have emerged to indicate that soybean MON 87705 is any less safe than its conventional counterpart and non-GM soybean reference varieties for the intended uses as proposed by the applicant.

As stated in the EFSA Guidance Document (2011), post-market monitoring should be required in specific cases, such as foods with altered nutritional composition and modified nutritional value and/or with specific health claims. A similar approach can apply to animal feed with altered nutritional characteristics.

Soybean MON 87705 does not raise toxicity or allergenicity concerns with respect to the insertion and expression of new traits, and the altered fatty acid profile in the context of the intended uses as compared with its conventional counterpart and non-GM soybean reference varieties. In addition, there were no indications of adverse effects after administration of diets containing defatted meal from soybean MON 87705 to rats.

The nutritional assessment was focused on the increased oleic acid and the accompanying decreased linoleic acid and palmitic acid content, of which the levels were outside the ranges of the measured

[24] Technical dossier/Section D7.10.2.
[25] The soybean reference varieties were Anand, Ozark, NK S38-T8, UA4805, NC+2A86 and NK25-J5.

biological variation. Total replacement of commercial soybean, canola and sunflower oils in margarine, salad dressing, mayonnaise and spread, and home-use liquid vegetable oil by oil derived from soybean MON 87705 would increase the intake of oleic acid and decrease the intake of linoleic and palmitic acid; these changes are in line with the current dietary recommendations and do not pose a nutritional concern. Therefore, the EFSA GMO Panel does not see the need for post-market monitoring.

The applicant has stated that soybean MON 87705 oil is targeted for applications such as margarine, salad dressing, mayonnaise and spread, and home-use liquid vegetable oil, but not for commercial frying (*i.e.* high-temperature and repeated frying).

### 5.2. Conclusion

No toxicity of the CP4 EPSPS protein was observed in an acute oral toxicity study in mice. The protein was rapidly degraded under simulated gastric conditions. In bioinformatics studies the protein showed no homology to known toxic proteins and allergens. A subchronic 90-day feeding study in rats using diets including defatted meal derived from soybean MON 87705 provided no indications of adverse effects. Testing of extracts from soybean MON 87705 and the conventional counterpart A3525 with sera from patients allergic to soybeans showed that the overall allergenicity of the whole plant had not been changed as a result of the genetic modification. A 42-day feeding study in broiler chickens demonstrated that diets formulated with defatted meal from soybean MON 87705 are as nutritious as diets with defatted meal from the conventional counterpart and commercial soybean reference varieties. The GMO Panel is of the opinion that soybean MON 87705 is as safe as its conventional counterpart and non-GM soybean reference varieties in the context of the intended uses as proposed by the applicant. The altered fatty acid profile did not raise concerns regarding toxicity.

The nutritional assessment is focused on the intended increase of oleic acid (C18:1) and the accompanying decreases of linoleic acid (C18:2) and palmitic acid (C16:0), of which the levels were outside the ranges of the natural variation. Changes in the levels of other fatty acids including the decrease of stearic acid (C18:0) and α-linolenic acid (C18:3) in MON 87705 compared with the conventional counterpart were small and the levels remained within the range of the fatty acid levels in the commercial reference varieties and described in the literature (see Table 2).

The slightly higher levels of the two minor fatty acids heptadecenoic acid (C17:1 9c) and octadecadienoic acid (C18:2 6c, 9c) in RBD oil from soybean MON 87705 (see section 5.1.2) do not raise concerns because of their low quantities and because they will be subject to normal fatty acid metabolic degradation.

The EFSA GMO Panel concludes that the estimated changes in fatty acid intake resulting from the replacement of conventional vegetable oils with oil from soybean MON 87705 do not raise nutritional concerns in the context of the intended use, as specified by the applicant, namely as margarine, salad dressing, mayonnaise, and home-use liquid vegetable oil. The EFSA GMO Panel is of the opinion that a specific nutritional assessment is required in case oil derived from soybean MON 87705 is used for food applications which have not been considered in the intake assessment provided to the Panel, *e.g.* commercial frying.

## 6. ENVIRONMENTAL RISK ASSESSMENT AND MONITORING PLAN

### 6.1. Environmental risk assessment

The scope of application EFSA-GMO-NL-2010-78 is for food and feed uses, import and processing and does not include cultivation. Considering the intended uses of soybean MON 87705, the environmental risk assessment is concerned with the exposure through the manure and faeces from animals fed soybean MON 87705 and with the accidental release into the environment of viable seeds of soybean MON 87705 during transport and processing.

Soybean MON 87705 was developed to have a modified fatty acid profile. Soybean MON 87705 also contains the *epsps* gene from *Agrobacterium* sp. CP4 to confer tolerance to glyphosate-containing herbicides.

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

### 6.1.1. Unintended effects on plant fitness due to the genetic modification[26]

Cultivated soybean species (*Glycine max* (L.) Merr.) belong to the subgenus *Soja* of the genus *Glycine*. The species originated from eastern Asia and is a highly domesticated crop (Lu, 2005). The major worldwide soybean producers are the United States (US), Brazil, Argentina, China, North Korea and South Korea. In the European Union, soybean is mainly cultivated in Italy, France and Romania (Dorokhov et al., 2004).[27]

Cultivated soybean seeds rarely display any dormancy characteristics and only under certain environmental conditions grow as volunteers in the year following cultivation (OECD, 2000). In soybean fields, seeds usually do not survive during the winter because of predation, rotting or germination resulting in death, or as a result of management practices prior to planting the subsequent crop (Owen, 2005).

The herbicide tolerance trait can be regarded as providing only a potential agronomic and selective advantage for this GM soybean plant where and when glyphosate-based herbicides are applied. The expected changes in seed fatty acid composition in soybean MON 87705 resulting from the introduced FAD2-1A/FATB1-A suppression cassette are not known to provide a potential agronomic and selective advantage. However, survival of soybean plants outside cultivation where glyphosate-based herbicides are applied is mainly limited by a combination of low competitiveness, absence of a dormancy phase and susceptibility to plant pathogens and cold climatic conditions. As these general characteristics are unchanged in soybean MON 87705, herbicide tolerance is not likely to provide a selective advantage outside cultivation where the herbicides are applied. Even if herbicides are applied to these plants, it will not change their ability to survive over seasons. Therefore, it is considered very unlikely that soybean MON 87705 will differ from conventional soybean varieties in its ability to survive until subsequent seasons or to establish feral populations under European environmental conditions.

Laboratory tests and field studies have been carried out to assess the phenotypic and agronomic characteristics as well as ecological interactions of GM soybean MON 87705 in comparison with an appropriate conventional counterpart with a similar genetic background and several non-GM soybean reference varieties as described in section 4.1.4. As mentioned above, some statistically significant differences were observed in the across location statistical analysis in the US field trials in 2007 and 2008 (in 2007 for early and final stand count, flowering and 100-seed weight, in 2008 for 100-seed weight). Soybean MON 87705 had a lower early and final stand count and a lower 100-seed weight than its conventional counterpart and the difference falls within the range of non-GM reference varieties. The observed differences are therefore unlikely to be biologically significant in terms of increased weed potential.

Germination and dormancy of seeds from MON 87705, control and reference varieties, produced in different environmental conditions, were evaluated in laboratory chambers through international protocols. Pollen characteristics were also assessed.

Considering the scope of the application, special attention is paid to those agronomic characteristics which may affect the survival, establishment and fitness of soybean MON 87705 seeds which could be

[26] Technical dossier/Sections D4, D9.1 and D9.2.
[27] http://epp.eurostat.ec.europa.eu/portal/page/portal/agriculture/data/database.

accidentally released into the environment: yield, plant height, shattering, germination, dormancy. There were no significant differences across field trials or laboratory experiments. Some site- or experiment-specific significant differences were observed but they were not indicative of a consistent plant response associated with the trait and, in most cases, they rather suggest a lower fitness of MON 87705 (lower germination capability and higher dead seed percentage for example). The EFSA GMO Panel considers that the differences observed are unlikely to affect the overall fitness, invasiveness or weediness of the GM soybean, except under conditions of application of glyphosate-based herbicides.

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 GM soybean and any change in its survival capacity, including overwintering (Dorokhov et al., 2004; Owen 2005; Bagavathiannan and Van Acker 2008, Lee et al., 2009). The EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects of the soybean MON 87705 in Europe will not be different from that of conventional soybean varieties.

### 6.1.2. Potential for gene transfer[28]

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

#### 6.1.2.1. Plant to bacteria gene transfer

Genomic DNA is a component of many food and feed products derived from soybean. It is well documented that DNA present in food and feed becomes substantially degraded during 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 animals feeding on soybean MON 87705 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 transfer 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 (HR). HR requires the presence of stretches of similar DNA sequences between 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.

Soybean MON 87705 contains the coding sequence for the enzyme CP4 EPSPS. The coding gene is a codon-optimised synthetic variant of the sequence of CP4 *epsps* from *Agrobacterium* sp. CP4. Other DNA fragments with sequence identity refer to four T-DNA regions between 20 and 275 bp in length which are located in flanking regions and within the insert containing the CP4 *epsps* cassette and the *FAD2-1A/FATB1-A* suppression cassette. Except the CP4 *epsps* gene and right and left border sequences of the Ti-plasmid, both closely related or identical to sequences of *A. tumefaciens*, none of the modified genetic elements of MON 87705 are of bacterial origin. *A. tumefaciens* can be isolated from soils, but 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 gene outside its

[28] Technical dossier/Section D9.2.

immediate receiving environment in the habitat of *A. tumefaciens* 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 MON 87705 or any other GM plant, it can be assumed that, as an extremely rare event, homologous recombination may occur between the recombinant CP4 *epsps* gene and *epsps* genes of *Agrobacterium* sp. CP4 or highly similar variants of this gene which may occur in other bacteria present in the environment (Kleter et al., 2005). Such recombination events would only replace natural variants (substitutive recombination) and are therefore 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 natural Ti-plasmids of *A. tumefaciens* would result in gene replacement, by which the recipient would lose its capability of crown gall formation (loss of auxin-, cytokinin- and opine-synthesising genes). The probability of this process is limited by the short lengths of the DNA-flanking regions providing DNA homologies (Brigulla and Wackernagel, 2010).

In addition to homology-based recombination processes, illegitimate recombination that does not require DNA similarity between the recombining DNA molecules is also theoretically possible. However, the transformation rates for illegitimate recombination are considered to be $10^{10}$-fold lower than for homologous recombination (Hülter, 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). Thus, this process, in comparison with homologous recombination, is not considered to significantly contribute to horizontal gene transfer events. In comparison with the above-described homology-facilitated recombination processes, the contribution of illegitimate recombination is extremely low.

The CP4 *epsps* gene of MON 87705 is regulated by a synthetic promoter (derived from the promoter of the figwort mosaic virus and the *Arabidopsis thaliana Tsf1* gene). The expression of the CP4 *epsps* construct in bacteria is unknown, but generally the expression level of eukaryotic promoters in bacteria is inefficient (Warren et al., 2008).

In a worst case scenario, considering the possibility of expression, a bacterial recipient would become capable of producing a plant-codon optimized CP4 EPSPS protein. The exposure of bacterial communities to the recombinant gene in soybean MON 87705 must, however, be seen in the context of the natural occurrence and level of exposure to alternative sources of similar genes to which bacterial communities are continually exposed. The protein encoded by CP4 *epsps* is an enzyme involved in the biosynthesis of chorismate, the common precursor of numerous aromatic compounds in bacteria, fungi and plants. It can therefore be expected that both sequence-similar and different *epsps* genes are widely distributed in gut and other environmental microorganisms. In the context of its intended use as food and feed, there is no direct exposure of *A. tumefaciens* to the herbicidal compound glyphosate. Therefore, the GMO Panel considers it unlikely that *A. tumefaciens* would gain a selective advantage by HGT from MON 87705.

The EFSA GMO Panel concludes that the CP4 *epsps* gene from soybean MON 87705 may, on a theoretical basis, replace highly similar genes by homologous recombination to *A. tumefaciens* or other bacteria. Owing to the natural occurrence of *epsps* genes or variants with high similarity in bacteria in the environment, a low level gene transfer or gene replacement in *A. tumefaciens* or other bacteria caused by MON 87705 is not regarded as conferring a new trait and selective advantage. Therefore, no risk connected to HGT from MON 87705 to bacteria has been identified by the EFSA GMO Panel.

6.1.2.2. Plant to plant gene transfer

Considering the intended uses of soybean MON 87705 and 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 transport and/or processing.

The genus *Glycine* is divided into two distinct subgenera: *Glycine* and *Soja*. Soybean is in the subgenus *Soja*. The subgenus *Glycine* contains 16 perennial wild species, while the cultivated soybean, *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*, *Glycine max* can cross only with other members of *Glycine* subgenus *Soja* (Hymowitz et al., 1998; Lu, 2005). Hence, the three species of the subgenus *Soja* are capable of cross-pollination and the hybrid seed that is produced can germinate normally and produce plants with fertile pollen and seed (Abe et al., 1999; Nakayama and Yamaguchi, 2002). However, since *Glycine soja* and *Glycine 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 transfer could therefore occur under the following scenario: imports of soybean MON 87705 seeds (although most MON 87705 seeds will be processed in the country 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 very close vicinity to cultivated soybean fields, overlap of flowering periods and 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, GM plants and plants derived from out-crossing with this GM soybean 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 because of predation, rotting or 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 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 87705 entering cultivation as this would require specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

In conclusion, as soybean MON 87705 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.

### 6.1.3. Interactions of the GM plant with target organisms[29]

Owing to the type of trait (changes in the fatty acid composition and herbicide tolerance with no target organisms) and the intended uses of soybean MON 87705, which exclude cultivation, this was not considered an issue by the EFSA GMO Panel.

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

Owing to the intended uses of soybean MON 87705, which exclude cultivation, and the low level of exposure to the environment, potential interactions of the GM plant with non-target organisms were not considered an issue by the EFSA GMO Panel.

### 6.1.5. Interactions with the abiotic environment and biogeochemical cycles[31]

Owing to the intended uses of soybean MON 87705, which exclude cultivation, and the low level of exposure to the environment, potential interactions with the abiotic environment and biogeochemical cycles were not considered an issue by the EFSA GMO Panel.

## 6.2. Post-market environmental monitoring[32]

The objectives of a monitoring plan according to Annex VII of Directive 2001/18/EC are (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the environmental risk assessment 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 environmental risk assessment.

Monitoring is also related to risk management, and thus a final adoption of the monitoring plan falls outside the mandate of EFSA. However, the EFSA GMO Panel gives its opinion on the scientific content of the monitoring plan provided by the applicant (EFSA, 2006b, 2011b). The potential exposure to the environment of soybean MON 87705 would be mainly through manure and faeces from animals fed with soybean MON 87705 or through accidental release into the environment of GM soybean seeds during transport and processing. The EFSA GMO Panel is aware that, owing to the physical characteristics of soybean seed and methods of transportation, accidental spillage cannot be excluded. Therefore, the EFSA GMO Panel recommends that appropriate management systems are introduced to actively monitor the occurrence of feral soybean plants in areas where soybean spillage and plant establishment are likely to occur as proposed in the EFSA Guidance Document (EFSA, 2006a, 2011a) and the scientific opinion of the EFSA GMO Panel on post-market environmental monitoring (EFSA, 2006b, 2011b).

The scope of the monitoring plan provided by the applicant is in line with the intended uses of the GMO. As the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects, no case-specific monitoring is necessary.

The general surveillance plan proposed by the applicant includes (1) the description of an approach involving operators (federations involved in soybean import and processing), reporting to the applicants, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment; (2) a coordinating system established by EuropaBio for the collection of information

[29] Technical dossier/Sections D8 and D9.4.
[30] Technical dossier/Section D9.5.
[31] Technical dossier/Sections D9.8 and D10.
[32] Technical dossier/Section D11.

recorded by the various operators (Lecoq et al., 2007; Windels et al., 2008); and (3) the use of networks of existing surveillance systems. The applicant proposes to submit a general surveillance report on an annual basis and a final report at the end of the consent.

Issues relating to the practical implementation of general surveillance and the evaluation of monitoring results are currently outside the remit of the EFSA GMO Panel. Details of the specific plans and methods of monitoring in each country should be developed by the applicant after the applications have been accepted (EFSA, 2006a,b, 2011a,b).

The EFSA GMO Panel is of the opinion that the scope of the monitoring plan proposed by the applicant is in line with the intended uses of soybean MON 87705 as the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan. 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 the case of accidental release of viable seeds of soybean MON 87705.

### 6.3. Conclusion

The scope of application EFSA-GMO-NL-2010-78 is for food and feed uses, import and processing and does not include cultivation. Considering the intended uses of soybean MON 87705, the environmental risk assessment is concerned with the indirect exposure mainly through manure and faeces from animals fed seed produced by soybean MON 87705 and with the accidental release into the environment of viable seeds produced by soybean MON 87705 during transport and processing.

In case of accidental release into the environment of viable seeds of soybean MON 87705 during transportation and processing, there are no indications of an increased likelihood of establishment and spread of feral soybean MON 87705 plants, except in the presence of glyphosate-based herbicides. In addition, the low levels of environmental exposure of these GM soybean plants and the newly expressed protein through other routes indicate that the risk to non-target organisms is extremely low. The EFSA GMO Panel considers that it is unlikely that the recombinant DNA in soybean MON 87705 transfers to bacteria. A risk caused by a rare but theoretically possible transfer of the recombinant genes from soybean MON 87705 to bacteria in the environment has not been identified by the GMO Panel because expression of these genes would not provide any selective advantage in the context of its intended use. The scope of the post-market environmental monitoring plan provided by the applicant and the reporting intervals are in line with the intended uses of soybean MON 87705.

## OVERALL CONCLUSIONS AND RECOMMENDATIONS

The molecular characterisation of soybean MON 87705 is considered sufficient and the EFSA GMO Panel considers that it does not indicate a safety issue.

The GMO Panel concludes, based on compositional, phenotypic and agronomic data, that soybean MON 87705 differs from the conventional counterpart and other non-GM soybean reference varieties only in the fatty acid profile and the newly expressed protein CP4 EPSPS.

The GMO panel concludes that (i) the altered fatty acid profile of oil from MON 87705 does not raise concerns regarding toxicity in the context of the intended uses, (ii) the CP4 EPSPS protein is not toxic, (iii) defatted soybean meal from MON 87705 was not toxic in an oral 90-day study in rats, (iv) the allergenicity of MON 87705 does not differ from that of its conventional counterpart and, finally, (v) soybean MON 87705 is as safe as its conventional counterpart and non-GM soybean reference varieties.

The nutritional assessment considered the intended increase of oleic acid and the accompanying decreases of linoleic and palmitic acid in the oil of MON 87705. Based on an exposure scenario with

the intended use of that oil in margarine, salad dressing, mayonnaise and spread, and for home-use, the GMO Panel concludes that the resulting changes in fatty acid intake do not raise nutritional concerns.

In the case of accidental release into the environment of viable seeds of soybean MON 87705 (*e.g.* during transport and processing), there are no indications of an increased likelihood of establishment and spread of feral soybean plants, except under application of glyphosate-based herbicides. The low levels of environmental exposure of these GM soybean plants indicate that the risk to non-target organisms is extremely low. The unlikely but theoretically possible transfer of the recombinant gene from soybean MON 87705 to environmental bacteria does not raise concern owing to the lack of a selective advantage in the context of its intended uses.

The scope of the post-market environmental monitoring plan provided by the applicant and the reporting intervals are in line with the intended uses of soybean MON 87705. 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 the case of accidental release of viable seeds of soybean MON 87705.

In conclusion, the EFSA GMO Panel considers that the information available for soybean MON 87705 addresses the scientific comments raised by Member States, and concludes considering the intended uses that the soybean MON 87705 is as safe as its conventional counterpart and is unlikely to have any adverse effect on human and animal health and the environment.

For specific labelling, the applicant proposed that, for example, operators handling products containing or consisting of oil produced from MON 87705 shall be required to label these products with the words "increased oleic acid oil produced from genetically modified soybean". The GMO Panel recommends adding the specific uses, *i.e.* "only for use in margarine, salad dressing, mayonnaise and spread, and for home-use".

## DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of The Netherlands, received 25 February 2010, concerning a request for placing on the market of Soybean MON 87705 submitted by Monsanto under Regulation (EC) No 1829/2003.

2. Acknowledgement letter, dated 10 March 2010, from EFSA to the Competent Authority of The Netherlands (Ref. PB/KL/shv (2010) 4697401).

3. Letter from EFSA to applicant, dated 31 March 2010, requesting additional information under completeness check (Ref. PB/CE/lg (2010) 4770695).

4. Letter from applicant to EFSA, received 30 June 2010, providing additional information under completeness check.

5. Letter from EFSA to applicant, dated 16 July 2010, requesting additional information under completeness check (Ref. PB/CE/lg (2010) 5003307).

6. Letter from applicant to EFSA, received 26 July 2010, providing additional information under completeness check.

7. Letter from EFSA to applicant, dated 13 August 2010, delivering the 'Statement of Validity' for application EFSA-GMO-NL-2010-78, Soybean MON 87705 submitted by Monsanto under Regulation (EC) No 1829/2003 (Ref. PB/KL/CE/shv (2010) 5052601 ).

8. Letter from EFSA to applicant, dated 5 November 2010, requesting additional information and stopping the clock (Ref. PB/KL/ZD/mt (2010) 5300701).

9. Letter from applicant to EFSA, received 17 January 2011, providing additional information.

10. Letter from EFSA to applicant, dated 3 February 2011, requesting additional information and maintaining the clock stopped (Ref. PB/KL/ZD/lg (2011) 5513031).

11. Letter from EFSA to applicant, dated 22 March 2011, requesting additional information and maintaining the clock stopped (Ref. PB/KL/ZD/shv (2011) 5635611).

12. Letter from applicant to EFSA, received 25 March 2011, providing additional information.

13. Letter from applicant to EFSA, received 21 June 2011, providing additional information.

14. Letter from EFSA to applicant, dated 2 August 2011, restarting the clock (Ref. PB/AFD/lg (2011) 5911854).

15. Letter from EFSA to applicant, dated 11 November 2011, requesting additional information and stopping the clock (Ref. EW/ZD/mt (2011) 6073285).

16. Letter from applicant to EFSA, received 29 December 2011, providing additional information.

17. Letter from EFSA to applicant, dated 27 February 2012, restarting the clock (Ref. EW/ZD/lg (2012) 6276051).

18. Letter from EFSA to applicant, dated 1 June 2012, requesting an updated Part IV (labelling proposal) and stopping the clock (Ref. EW/ZD/CE/lg (2012) 6615601).

19. Letter from applicant to EFSA, received 5 June 2012, providing the requested updated Part IV.

20. Letter from EFSA to applicant, dated 8 June 2012, restarting the clock (Ref. EW/ZD/lg (2012) 6637369).

21. Letter from applicant to EFSA, dated 4 October 2012, providing the Certificate of Analysis for the AOCS Certified Reference Material.

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