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SCIENTIFIC OPINION

Scientific Opinion on application (EFSA-GMO-BE-2010-79) for the placing on the market of insect resistant genetically modified soybean MON 87701 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 an evaluation of a risk assessment of the genetically modified, insect resistant, soybean MON 87701 for food and feed uses, import and processing. Soybean MON 87701 was developed through *Agrobacterium*-mediated transformation. It contains a single insert consisting of a *cry1Ac* expression cassette, encoding the Cry1Ac protein that confers resistance against specific lepidopteran insects. The stability of the insert was confirmed over multiple generations. Bioinformatic analyses of the insert and its flanking regions, and levels of newly expressed protein did not raise safety concerns. Comparative analyses of compositional, phenotypic and agronomic characteristics indicated that soybean MON 87701 is not different from its conventional counterpart (A5547) and equivalent to commercial soybean varieties, except for having an increased vitamin E content (still within normal ranges) and expressing the Cry1Ac protein. The safety assessment of the Cry1Ac protein and soybean MON 87701 identified no concerns regarding potential toxicity and allergenicity. A feeding study on broiler chickens confirmed that defatted soybean MON 87701 meal is as nutritious as conventional defatted soybean meal. There are no indications of an increased likelihood of establishment and spread of feral soybean plants. Considering its intended use as food and feed, environmental risks associated of an unlikely but theoretically possible horizontal gene transfer from soybean MON 87701 to bacteria have not been identified. Potential interactions of soybean MON 87701 with the biotic and abiotic environment were not considered due to the low level of exposure. The monitoring plan and reporting intervals are in line with the intended uses of soybean MON 87701. The EFSA GMO Panel considers that the soybean MON 87701, as described in this 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.

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[1] On request from the Competent Authority of Belgium for an application (EFSA-GMO-BE-2010-79) submitted by Monsanto, Question No EFSA-Q-2010-00867, adopted on 6 July 2011.

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KEY WORDS

GMO, soybean (*Glycine max*), MON 87701, insect resistant, Cry1Ac, human and animal health, import and processing, Regulation (EC) No 1829/2003

SUMMARY

Following the submission of an application (EFSA-GMO-BE-2010-79) 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 insect resistant genetically modified (GM) soybean MON 87701 (Unique Identifier MON-877Ø1-2) for food and feed uses, import and processing.

In delivering its scientific opinion, the EFSA GMO Panel considered the application EFSA-GMO-BE-2010-79, additional information supplied by the applicant, scientific comments submitted by the Member States, and relevant scientific publications. The scope of application EFSA-GMO-BE-2010-79 is for food and feed uses, import and processing of soybean MON 87701 within the European Union as any non-GM soybean but excludes cultivation in the EU. The EFSA GMO Panel evaluated soybean MON 87701 with reference to the intended uses 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). The scientific evaluation of the risk assessment included molecular characterisation of the inserted DNA and expression of the corresponding proteins. An evaluation of the comparative analysis of composition, phenotypic and agronomic characteristics 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 were undertaken.

Soybean MON 87701 was transformed using *Agrobacterium tumefaciens*. Soybean MON 87701 expresses the *cry1Ac* gene leading to the production of the Cry1Ac insecticidal crystal protein (δ-endotoxin). The Cry1Ac protein provides protection from feeding damage caused by specific lepidopteran pests in the soybean.

The molecular characterisation data establish that the genetically modified soybean MON 87701 contains one copy of an intact *cry1Ac* expression cassette. 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 spanning the newly created DNA junctions did not indicate any safety concern. The stability of the inserted DNA was confirmed over several generations and a Mendelian inheritance pattern was demonstrated.

The EFSA GMO Panel compared the composition, phenotype and agronomic characteristics of soybean MON 87701 and its conventional counterpart (A5547), assessed all statistical differences identified, and came to the conclusion that soybean MON 87701 is compositionally not different from its conventional counterpart except for having an increased vitamin E content (still within the normal range of soybeans) and expressing the Cry1Ac protein. Except for expressing the Cry1Ac protein, soybean MON 87701 is also compositionally and agronomically equivalent to commercial soybean varieties. The risk assessment of the newly expressed protein and the whole crop included an analysis of data from analytical and bioinformatics studies, as well as in vitro and in vivo studies. The EFSA GMO Panel concluded that the soybean MON 87701 is as safe as its conventional counterpart and that the overall allergenicity of the whole plant is not changed.

The application EFSA-GMO-BE-2010-79 concerns food and feed uses, import, and processing. Therefore, there is no requirement for scientific information on possible environmental effects associated with the cultivation of soybean MON 87701. There are no indications of an increased likelihood of establishment and spread of feral soybean plants in case of accidental release into the environment of viable soybean MON 87701 grains during transportation and processing for food and feed uses. Taking into account the scope of the application, both the rare occurrence of feral soybean plants and the low levels of exposure through other routes indicate that the risk to target and non-

target organisms is extremely low. The unlikely but theoretically possible transfer of the recombinant gene from soybean MON 87701 to environmental bacteria does not raise concern due 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 is in line with the intended uses of soybean MON 87701. Furthermore, the EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

In conclusion, the EFSA GMO Panel considers that the information available for soybean MON 87701 addresses the scientific issues indicated by the Guidance document of the EFSA GMO Panel and the scientific comments raised by the Member States, and that the soybean MON 87701 is as safe as its conventional counterpart with respect to potential effects on human and animal health or the environment in the context of its intended uses.

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BACKGROUND

On 17 May 2010, the European Food Safety Authority received from the Belgian Competent Authority an application (Reference EFSA-GMO-BE-2010-79) for authorisation of genetically modified (GM) soybean MON 87701 (Unique Identifier MON-877Ø1-2) submitted by Monsanto within the framework of Regulation (EC) No 1829/2003 on GM food and feed[4]. After receiving the application EFSA-GMO-BE-2010-79 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 11 June 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 11 September 2010) within which to make their opinion known.

The Scientific Panel on Genetically Modified Organisms of EFSA (EFSA GMO Panel) carried out an evaluation of the scientific risk assessment of the GM soybean MON 87701 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 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). 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 21 June 2010, 15 November 2010, and 04 March 2011, the EFSA GMO Panel requested additional information from the applicant. The applicant provided the requested information on 01 July 2010, 03 January 2011, and 15 March 2011.

In giving its opinion on soybean MON 87701 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 87701 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation

[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.

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

The EFSA GMO Panel was not requested to give a scientific opinion on information required under Annex II to the Cartagena Protocol. Furthermore, the EFSA GMO Panel did also not consider proposals for labelling and methods of detection (including sampling and the identification of the specific transformation event in the food/feed and/or food/feed produced from it), which are matters related to risk management.

ASSESSMENT

1. Introduction

The GM soybean MON 87701 (Unique Identifier MON-877Ø1-2) was evaluated with reference to its intended uses, taking account of the appropriate principles described in the 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). The evaluation of the 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.

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]

Meristem tissue excised from embryos of germinated seeds of conventional soybean A5547 was transformed with the binary plasmid PV-GMIR9 using *Agrobacterium tumefaciens* (renamed *Rhizobium radiobacter*) strain ABI. The plasmid PV-GMIR9 contained two T-DNAs. T-DNA I contained the *cry1Ac* expression cassette that provides insecticidal activity against specific lepidopteran insects. T-DNA II contained the CP4 *epsps* cassette conferring tolerance to glyphosate, which in this case served as the selectable marker for transformation. The two-T-DNA system utilised here enabled the cassettes encoding the trait of interest and the selectable marker to be inserted at two independent genetic loci within the genome of the plant. Transformants were selected with glyphosate and shoot formation was induced without callus phase. After self-pollination of the transformed R_0 plant, an R_1 plant (designated as MON 87701) that contained a single T-DNA I but did not contain T-DNA II was selected for further development.

The two T-DNA cassettes present in plasmid PV-GMIR9 consisted of the following elements between their respective right and left border regions:

T-DNA I (*cry1Ac* expression cassette): (1) promoter and 5' non-translated region of the *Arabidopsis thaliana rbcS4* gene (*rbcS4* gene encodes ribulose 1,5-bisphosphate carboxylase (Rubisco) small subunit 1A) to provide expression in the photosynthetic tissues; (2) sequence encoding the transit peptide of *rbcS4* gene to target the protein to the chloroplast; (3) modified coding sequence of the *cry1Ac* gene of *Bacillus thuringiensis* to confer resistance to specific lepidopteran insects; (4) 3' region of soybean *sphas1* gene (*sphas1* gene encodes β-conglycinin, a 7S α' seed storage protein), including 35 nucleotides of the carboxy-terminus of β-conglycinin coding region, termination codon and polyadenylation sequence. The *cry1Ac* gene of *B. thuringiensis* was modified by site-directed mutagenesis to increase its expression in the plant. The amino acid sequence of the processed protein in the plant is nearly identical (>99 %) to that of *B. thuringiensis*, with seven amino acid differences

[6] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2010-00867
[7] Technical dossier/sections C and D1

which falls within the normal variation among Cry1Ac proteins. In addition there are four additional amino acids in the N-terminus derived from the chloroplast transit peptide.

T-DNA II (CP4 *epsps* expression cassette): (1) FMV promoter from Figwort Mosaic Virus 35S RNA gene, which drives transcription in most plant cells; 5' non-translated leader sequence from the *Arabidopsis shkG* gene (*shkG* gene encodes EPSPS) to enhance expression; (2) sequence encoding the transit peptide of *shkG* gene to target the protein to the chloroplast; (3) modified coding sequence of the *aroA* gene from *A. tumefaciens* strain CP4 encoding the EPSPS protein to confer tolerance to glyphosate during the selection of transformants; (4) 3' non-translated transcriptional termination sequence and polyadenylation signal sequence from pea *rbcS2* gene *E9* (*rbcS2* gene encodes Rubisco small subunit).

Additional functional elements in the plasmid vector outside of the T-DNAs, and thus not expected to be transferred to the soybean genome, were: (1) *oriV* origin of replication to maintain the plasmid in *Agrobacterium*; (2) *ori-pBR322* origin of replication to maintain the plasmid in *Escherichia coli*; (3) *rop* repressor of primer (ROP) protein to maintain plasmid copy number in *E. coli*; (4) *aad*A bacterial selectable marker (promoter and coding regions) to confer spectinomycin/streptomycin resistance.

3.1.2. Transgene constructs in the genetically modified plant[8]

Molecular analyses indicated that the GM soybean MON 87701 contains a single insert with one copy of the intact *cry1Ac* expression cassette. No elements from the T-DNA II or vector backbone were detected. Southern analyses of genomic DNA from soybean MON 87701 and its non-GM counterpart A5547 were performed using appropriate combinations of restriction endonucleases and eleven overlapping probes that cover the whole plasmid. The probes corresponding to the different elements of T-DNA I showed the expected hybridisation signals, whereas no signal was observed for any of the probes corresponding to the vector backbone of PV-GMIR9, including T-DNA II. Some probes detected endogenous soybean sequences as part of T-DNA I was originally isolated from soybean.

The nucleotide sequence of the insert as well as both 5' and 3' flanking regions were determined from soybean MON 87701. This confirmed the conclusions drawn from the Southern analyses. Comparison to the parental soybean A5547 indicated that in soybean MON 87701, a 32 bp DNA segment of endogenous DNA had been deleted and 14 bp have been introduced immediately 5' of the insertion site.

To determine the possible disruption of known endogenous soybean genes by the insertion in soybean MON 87701, bioinformatic analyses were carried out on the genomic sequences flanking the insert (c.a. 1.5 kb on each side of the insert). In addition, the possible presence of coding sequences in the soybean genome flanking the insert was analysed. BLASTN searches were performed against EST (Expressed Sequence Tag) database and non-redundant nucleotide database and BLASTX search against non-redundant amino acid database. The results did not indicate the interruption of a soybean coding sequence(s) with known function in the MON 87701 event.

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

3.1.3.1. Expression of the introduced gene

Cry1Ac levels were analysed by enzyme-linked immunosorbent assay (ELISA) from a number of plant parts including root, leaf, seed and forage, from replicated field trials across five locations in the

[8] Technical dossier/section D2
[9] Technical dossier/section D3

US (2007) and five locations in Argentina (2007-2008). Considering the scope of the application, the Cry1Ac protein levels in seeds are considered most relevant. In 2007 US growing season the mean level was 4.7 µg/g dry weight (dw) and range 3.4–5.7 µg/g dw. In 2007-2008 Argentina growing season the mean level was 5.1 µg/g dw and range 3.9-6.7 µg/g dw.

3.1.3.2. Putative cryptic open reading frames in soybean MON 87701

Bioinformatic analyses were performed on hypothetical polypeptides encoded by the 5' and 3' junctions between the insert and soybean genomic DNA as well as on the open reading frames of the entire insert. The purpose was to predict the expression of intended or unintended novel (poly)peptides with toxic or allergenic properties or other adverse biological activity. DNA sequences were translated in all six reading frames. Each translated sequence was compared to protein databases, including allergen sequence database, toxin sequence database and a database containing sequences of all known proteins. The FASTA analyses included both overall sequence alignments as well as searches for short identical stretches of at least eight contiguous amino acids against the allergen database. No alignment met or exceeded the Codex Alimentarius (2009) threshold for potential allergenicity, and no relevant similarities to known toxic proteins other than Bt proteins (Cry1Ac) were found.

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

Genetic stability of the inserted DNA was studied by Southern analysis from five generations, all of them produced by self-pollination. The restriction enzyme/probe combinations used were sufficient to conclude that all the generations tested retained only a single copy which was stably inherited in subsequent generations.

Stability was also demonstrated by testing the presence of the Cry1Ac protein (by ELISA or lateral flow strips) or the *cry1Ac* gene (real-time PCR) over several generations produced by self-pollination. Furthermore, plants of F_2 and F_3 generations, derived from soybean MON 87701 (R_5 generation) back-crossed with a conventional soybean variety, were analysed by event-specific real-time PCR for the presence of the *cry1Ac* gene and the results were subjected to segregation analysis. In total nearly 2000 plants were tested. The results confirmed that the *cry1Ac* gene was stably inherited and followed a Mendelian segregation pattern.

3.2. Conclusion

The molecular characterisation data establish that the GM soybean MON 87701 contains one copy of an intact *cry1Ac* expression cassette. 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 spanning the newly created DNA junctions did not indicate any safety concern. The stability of the inserted DNA was confirmed over several generations and a Mendelian inheritance pattern was demonstrated.

[10] Technical dossier/section D5

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]

The application EFSA-GMO-BE-2010-79 for food and feed use, import and processing of soybean MON 87701 within the European Union presented compositional data of seed and forage material of soybean MON 87701 collected in field trials in the USA in 2007 and in Argentina in the season 2007/2008. The results of these studies have recently been published (Berman et al., 2009). These field trials compared the composition of soybean MON 87701 with a soybean conventional counterpart having a comparable genetic background. The conventional counterpart was the non-transgenic Asgrow variety A5547, which was the commercial soybean variety originally used when the soybean was transformed to establish the MON 87701 event.[12]

In both years/seasons the field trials were performed at five different sites, all of which are representative for soybean cultivation areas in the USA and Argentina, respectively. Each field trial included soybean MON 87701, the conventional counterpart (A5547) and four different commercial non-GM soybean varieties per field trial site, all being treated with pesticides according to conventional practice. Overall, 20 commercial soybean varieties were used as reference lines aimed at providing data on the natural variation in composition of this food and feed plant. The reference lines were characterized by event-specific PCR analysis for the presence or absence of MON 87701. Samples of one of the replicates of MON 87701 from one of the trial sites and of one of the replicates of A5547 from another site were found to be contaminated with GM material and were excluded from the study. At each trial site, soybean MON 87701, the conventional counterpart and the reference lines were planted following a randomized complete block design with three replicates at each site. Whereas all replicates of soybean MON 87701 and its conventional counterpart were chemically analysed for selected soybean constituents, only one of the replicates of the reference lines was analysed for these constituents.

4.1.2. Compositional analysis[13]

Soybean seeds were harvested and analysed for proximates (protein, fat, ash, and moisture), carbohydrates by calculation, fiber fractions (acid detergent fiber (ADF) and neutral detergent fiber (NDF)), amino acids, fatty acids, vitamin E, anti-nutrients (i.e. phytic acid, trypsin inhibitor, lectins, stachyose and raffinose) and other secondary metabolites (isoflavones). Forage was analysed for proximates, carbohydrates by calculation, and fibre fractions (ADF, NDF). In total, 64 different compounds were analysed, 57 in seeds and seven in forage, essentially those recommended by OECD (2001). The data on each compound were statistically analysed for potential differences in levels between soybean MON 87701 and its conventional counterpart within-site and across-sites (sites of the trial combined). Nine of the fatty acids analysed in material from the field trials in the USA and 11 in the material from Argentina were rare and often found at levels below the limit of quantification; when this occurred in more than 50 % of the samples, the analyte in question was excluded from the statistical analysis. When the value for a given compound was statistically different between soybean MON 87701 and its conventional counterpart, such value was compared to those occurring in the commercial soybean varieties included in the study, as well as to the ranges in the level of the compound in soybean published in the scientific literature and the ILSI crop composition database (Ridley et al., 2004).

[11] Technical dossier/section D7.2
[12] Technical dossier/section C1
[13] Technical dossier/section D7.1

When the compositional data for seed samples from the field trials in the USA were statistically evaluated across sites, statistically significant differences between soybean MON 87701 and its conventional counterpart were found for 15 analytes: the proximates protein and carbohydrates; the amino acids alanine, glycine, histidine, isoleucine, leucine, lysine, serine, threonine, and valine; the fatty acid 22:0 behenic acid; vitamin E, trypsin inhibitor, and daidzein. For forage, none of the analysed compounds showed significant differences between soybean MON 87701 and its conventional counterpart. The evaluation of the compositional data for seed samples per site revealed that, out of the 15 analytes found to be significantly different, five were significantly different at one individual field trial sites, three at two sites, and one at four sites. The statistically significant differences found were usually small. Apparently, the increase in the nine amino acids identified reflected the increased protein content of the seed. Some inconsistent changes in daidzein levels were noted. The only case where the difference was appreciable was vitamin E (23.2 % increase); the vitamin E level was significantly higher in soybean MON 87701 than in its conventional counterpart at four of the five field trial sites (17-37 %). In all cases except one, the level of vitamin E and all other measured compounds were within the range defined by the commercial reference varieties included in the study and reported by the ILSI crop composition database (Ridley et al., 2004) or the USDA-ISO (2006) isoflavone database. The exception was a single calculated carbohydrate value for soybean MON 87701, found to be slightly below the range defined by the commercial soybean varieties, which did not raise concern for the EFSA GMO Panel. Also, the 17 additional statistically significant differences (not significant in the overall analysis) identified in the per location analysis of other soybean constituents were small and within the range defined by the commercial reference varieties included in the field trials.

The statistical evaluation of compositional data of seed samples across sites of Argentinean field trials, revealed a statistically significant difference between soybean MON 87701 and its conventional counterpart for four analytes: the amino acid tryptophan, the fatty acid 18:3 linolenic, vitamin E, and stachyose. For forage, none of the analysed parameters differed significantly between soybean MON 87701 and its conventional counterpart. The evaluation per site revealed that, of these four constituents, tryptophan was significantly different at two individual field trial sites, linolenic acid at three sites, vitamin E at all five sites, and stachyose at none of the five sites. The statistically significant differences found between soybean MON 87701 and its conventional counterpart were usually small, and in all cases the levels registered were within the range defined by the commercial reference varieties included in the study and reported by the ILSI crop composition database or the USDA-ISO isoflavone database. Also, the nine additional statistically significant differences identified in the per location analysis of other soybean constituents were small, and all but one (moisture content in seeds of the control material) were within the range defined by the 20 soybean reference varieties.

Thus, only the vitamin E level was significantly different between seeds of soybean MON 87701 and its conventional counterpart when statistically analysed across field trial sites both in 2007 (7.69 vs 6.24 mg/100g dry weight) and the season 2007/2008 (4.40 vs 3.42 mg/100g d.w.). Analysis per site revealed increased vitamin E levels at nine of the 10 field trial sites studied. The EFSA GMO Panel concludes that the vitamin E level is increased (on average around 25 %) in soybean MON 87701 but that the level still is within the range of values commonly observed in conventional commercial soybean varieties, as defined by the reference lines, and by the ILSI crop composition database.

Berman et al. (2010) recently published compositional data on the soybean event MON 87701 grown at two field trial sites in Southern Brazil and at two field trial sites in Northern Brazil in 2007/2008. Whereas the MON 87701 event occurred in the A5547 genetic background in field trials performed in Southern Brazil, it occurred in the Monsoy 8329 background in the northern field trials. These studies confirmed the compositional information obtained from the field trials in the USA and Argentina. Hierarchical cluster analysis and principal component analysis of the Brazilian data further showed that location (site and region) and/or germplasm (genetic background) effects contributed more to the compositional differences between soybean MON 87701 and its conventional counterparts than the genetic modification.

The EFSA GMO Panel considered the total set of compositional data supplied and the statistically significant differences between soybean MON 87701 and its conventional counterpart in the light of the field trial design, measured biological variation and the level of the studied compounds in commercial soybean varieties, and concludes that soybean MON 87701 is compositionally not different from its conventional counterpart soybean A5547 except for having an increased vitamin E content (still within the normal range of soybeans) and expressing the Cry1Ac protein. Except for expressing the Cry1Ac protein, soybean MON 87701 is compositionally equivalent to commercial soybean varieties.

4.1.3. Agronomic traits and GM phenotype[14]

Based on data collected at 16 field trial sites in the USA in 2007 and 8 field trial sites in Argentina during the season 2007/2008, the applicant performed a comparative assessment of the phenotypic and agronomic characteristics of soybean MON 87701 and its conventional counterpart (A5547). A randomized complete block design was used at each field trial site. These field trials also included several commercial soybean varieties (four per site) used as reference material to estimate the range in baseline values for the studied phenotypic and agronomic parameters in commercial soybean varieties. All materials were grown under normal agronomic conditions for the geographical region; all maintenance chemicals were commercially registered products and were applied at recommended rates. The phenotypic and agronomic characteristics evaluated were early stand count, seedling vigour, plant growth stages, days to 50 % flowering, flower colour, plant pubescence, plant height, lodging, pod shattering, final stand count, seed moisture, 100 seed weight, test weight (g/250 ml), and yield. Seed dormancy and germination, and pollen characteristics were also considered.

In the field trials performed in the USA, no significant differences were detected between soybean MON 87701 and the conventional counterpart regarding the phenotypic and agronomic parameters investigated. In the field trials performed in Argentina early stand count (96.9 vs 105.9 plants in defined rows) and seed moisture were reduced, and test weight increased. Differences observed for early stand count, seed moisture and test weight were observed in two, five and three out of eight sites respectively. Whereas seed moisture and the test weight were within the range of values defined by the reference soybean varieties, the early stand count for soybean MON 87701 (96.9 plants) were slightly below the range for the reference varieties (103.8-204.0 plants). The applicant suggested that the lower early stand count could be due to different climatic conditions during production of the seeds used for the present field trials, as differences in field emergence due to climatic factor have been observed for soybean varieties with reduced raffinose content (Meis et al., 2003). The reference seeds used in the present study were produced under commercial production practices in a temperate climate in the USA while the MON 87701 seeds were produced in a subtropical environment in Puerto Rico. The EFSA GMO Panel found the explanation acceptable. As indicated above, the reduced early stand count did not influence yield and final stand count.

Specific studies were performed on pollen morphology and viability. There was no difference in percent viable pollen, pollen diameter and pollen morphology between soybean MON 87701 and the conventional counterpart. Seed germination and dormancy characteristics were evaluated on soybean seeds obtained from one field trial site. Whereas no hard seeds were detected in soybean MON 87701 at a temperature of 20^{o} C, 0.5 % of the A5547 seeds were hard at this temperature. As this difference in seed hardness was within the range of soybean reference varieties and no difference was detected at other temperatures, the EFSA GMO Panel did not find this observation as indicating relevant alterations in germination characteristics.

The reference material included in some of the field trials to provide estimates of the natural variation in studied phenotypic and agronomic characteristics was a mixture of GM and non-GM soybean varieties. Therefore, the EFSA GMO Panel asked the applicant to provide ranges in the levels of

[14] Technical dossier/section D4

studied parameters based on only the non-GM reference varieties. Relevant ranges were provided and showed that the original conclusions were confirmed by the new analysis.[15] The EFSA GMO Panel concludes that the field studies on agronomic performance and phenotypic characteristics identified no difference between soybean MON 87701 and its conventional counterpart that are likely to be biologically relevant. Levels in parameters that showed a statistically significant difference between soybeans MON 87701 and A5547 fell, except for early stand count, within the range in levels defined by a set of reference soybean varieties describing the natural variation.

4.2. Conclusion

Based on the comparative analysis of soybean MON 87701, the conventional counterpart and several other commercial non-GM soybean varieties, the EFSA GMO Panel concludes that soybean MON 87701, as assessed in this application, is compositionally, phenotypically and agronomically not different from its conventional counterpart except for expressing the Cry1Ac protein and showing increased levels of vitamin E, and equivalent to commercial soybean varieties, except for the presence of the newly expressed protein (Cry1Ac).

5. Food/feed safety assessment

5.1. Evaluation of relevant scientific data

5.1.1. Product description and intended use[16]

The scope of application EFSA-GMO-BE-2010-79 is for food and feed use, import and processing of soybean MON 87701 within the European Union. Thus, soybean MON 87701 will be imported into the EU mixed with other soybean varieties and be used as food or feed, or for the production of a large number of derived products, as any commercial soybean variety. The main product for human use is soybean oil. Around 10 % of the heat-processed (toasted) defatted soybean meal goes to production of soybean products for human consumption, including flours, soybean protein concentrates and various textured products simulating meats, sea-foods and cheeses. The rest of the toasted defatted soybean meal goes to feed, in the European Union mainly to poultry, pig and cattle (OECD, 2001). Whole soybeans are used to produce soy sprouts, baked soybeans, and roasted soybeans. There is also a limited direct use of soybeans as animal feeds.

The genetic modification event present in soybean MON 87701 results in the expression of a new protein, the Cry1Ac protein, that confers protection against lepidopteran pests such as velvet bean caterpillar (*Anticarsia gemmatalis*), soybean looper (*Pseudoplusia includens*), soybean anxil borer (*Epinotia aporema*) and sunflower looper (*Rachiplusia nu*). Thus, the genetic modification is intended to improve agronomic performance only and is not intended to influence the nutritional characteristics, the processing characteristics and overall use of soybean as a crop.

5.1.2. Effects of processing[17]

Soybean MON 87701 will be used for production and manufacturing of food and feed products as any other commercial soybean variety. Taking into account the compositional analysis, providing no indication of relevant compositional changes, the EFSA GMO Panel has no reason to assume that the

[15] Additional information July 2011
[16] Technical dossier/section D7.7
[17] Technical dossier/section D7.6

characteristics of soybean MON 87701 and derived processed products would be different from those of the respective products derived from conventional soybean varieties.

Soybean MON 87701 will be processed in the same manner as conventional soybeans. A heat treatment of soybean MON 87701 (190°C for 15.5 min) reduced the quantity of immunodetectable Cry1Ac protein to levels below the limit of detection, corresponding to a 94 % reduction compared to the level in non-treated MON 87701. It was suggested that the heat-induced losses likely are due to protein degradation and/or aggregation of the Cry1Ac proteins into insoluble complexes.

5.1.3. Toxicology[18]

5.1.3.1. Protein used for safety assessment

Due to the relatively low expression level of the Cry1Ac protein in soybean MON 87701 (see section 3.1.3.), and the very difficult task to isolate a sufficient quantity of purified protein from the GM soybean, the safety studies with the newly expressed protein were conducted with a Cry1Ac protein encoded by the *cry1Ac* gene from a specific strain of *B. thuringiensis* and expressed in *E. coli*. The structural similarity and physicochemical and functional equivalence of the Cry1Ac protein produced by *E. coli* to that produced in soybean MON 87701 was shown by N-terminal sequencing (Edman degradation), Western analysis with Cry1Ac specific antibodies, mobility in SDS-PAGE, proteolytic peptide mapping following MALDI-TOF mass spectrometry, glycosylation analysis and Cry1Ac biological activity. Together, these methods confirmed the equivalence of the bacterial and the plant Cry1Ac proteins. Based on the identified similarity in structure and equivalence in physico-chemistry and function between these proteins, the EFSA GMO Panel accepts the use of a Cry1Ac test material derived from *E. coli* for the degradation studies and safety testing of the Cry1Ac protein present in soybean MON 87701, as well as for use as a reference standard in the ELISA to estimate Cry1Ac expression levels in various tissues of soybean MON 87701.

5.1.3.2. Toxicological assessment of the expressed novel protein in soybean MON 87701

The newly introduced gene in soybean MON 87701 is derived from the soil bacterium *Bacillus thuringiensis* subsp. *kurstaki*. The gene codes for a protein, Cry1Ac, which is insecticidal specifically against lepidopteran insects but is unknown to be toxic to humans and animals. Products of cotton MON 531, expressing a Cry1Ac protein that in amino acid sequence is 100 % identical to the protein expressed in soybean MON 87701, but contains four additional amino acids at the N-terminus of the MON 87701-produced protein, has been on the European market as existing food or feed, and food additive since the end of 2002, without any adverse effects to human health having been reported.

(a) Acute toxicity testing

The Cry1Ac protein produced in a recombinant *E. coli* strain did not induced adverse effects in an acute oral toxicity study in CD-1 mice administered a single dose of 1460 and 1290 mg/kg body weight to male and female animals, respectively.

(b) Degradation in simulated digestive fluids

Digestion of the Cry1Ac protein (1182 amino acids) in a pepsin digestion assay (simulated gastric fluid) was studied *in vitro* by identifying peptide fragments using SDS-PAGE colloidal blue gel staining and Western analysis. The SDS-PAGE colloidal blue gel staining demonstrated that at least 99.7 % of the Cry1Ac protein produced in *E. coli* was fully degraded by pepsin-containing simulated

[18] Technical dossier/section D7.8

gastric fluid of pH 1.2 within 30 seconds. In agreement with this finding, Western analysis showed that most (>95 %) of the Cry1Ac protein was digested in simulated gastric fluid within the same time frame. The digestion resulted in fragments (3.5-4 kDa) of the Cry1Ac protein visible for up to 60 min on the gel. These fragments of the Cry1Ac protein started at amino acid positions 415 and 882, respectively. Combining a two minute exposure of the Cry1Ac protein to simulated gastric fluid (producing complete degradation of the full length protein and appearance of the short fragment) with a subsequent exposure of the digest to simulated intestinal fluid (neutral pH, 1 min), resulted in complete disappearance of the shorter fragment. In simulated intestinal fluid, the full length Cry1Ac was digested below the limit of detection within five minutes, producing a trypsin-resistant core polypeptide (around 55 kDa) stable throughout the digestion.

(c) Bioinformatics studies

Searches for amino acid sequence homology of the Cry1Ac protein expressed in soybean MON 87701 with amino acid sequences of toxic proteins stored in an updated propriety data base using the FASTA sequence alignment tool, indicated significant homology only with other Cry proteins not toxic to humans and animals. Thus, no safety concerns for humans and animals were identified.

5.1.3.3. Toxicological assessment of new constituents other than proteins

No new constituent other than the Cry1Ac protein is expressed in soybean MON 87701 and no relevant changes in the composition of soybean MON 87701 were detected by the compositional analysis.

5.1.3.4. Toxicological assessment of the whole GM food/feed

No indication was found in the molecular analysis and in the comparative compositional, phenotypic and agronomic analysis that the genetic modification of soybean MON 87701 resulted in any unintended changes. According to the 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), animal safety studies with the whole food/feed are not required in these cases .

However, the applicant supplied a 90-day feeding trial with Sprague-Dawley rats of the Crl:CD strain. This study was performed using a protocol adapted from OECD Guideline 408 (OECD, 1998), with 12 animals per sex and treatment. Diets were provided ad libitum and contained 30 % toasted and defatted soybean meal prepared from either soybean MON 87701, the conventional counterpart (A5547), or one of three conventional reference soybean varieties: Anand, UA4805 or Ozark. Diets were nutritionally balanced and analysed for its composition, in particular in relation to feed quality.

There were no differences in mean weekly body-weights and cumulative mean body weight gains between male animals of the group fed diets with processed MON 87701 soybean meal as compared to male rats administered diets with processed meal of soybean A5547. However, the mean weekly body-weights and cumulative body weight gains of female rats given the MON 87701-supplemented diet were consistently, and, in particular, from week 9 onwards, statistically significantly lower than in female rats administered diets with processed A5547 soybean meal. The study was confounded by the fact that the mean body-weight of the female rats receiving the MON 87701-based diet was already lower at the start of the study. Feed consumption was generally lower for female rats given the MON 87701-containing diet.

The applicant concluded that the observed differences in female body-weight and cumulative body-weight gain most likely were attributable to biological variation, and were not treatment-related. To confirm this interpretation of the result of the initial study (subsequently called study I), the applicant performed a second 90-day feeding study (study II) in rats. The second study used 20 rats/sex/group, and 15 % and 30 % incorporation of processed MON 87701 or A5547 soybean meal in the diet.

In study II, there were no relevant differences in body-weight, cumulative body-weight gain and feed intake in female rats receiving the MON 87701-containing diets as compared to females administered the A5547-containing diet (control diet). However, in this study the mean body-weight and cumulative body-weight gains in male rats receiving 15 % and 30 % MON 87701 soybean meal in the diet were statistically significantly higher than in rats receiving the control diets throughout a considerable part of the study. A statistically significantly higher feed consumption was observed during specific time periods in male animals receiving 15 % and 30 % MON 87701 soybean meal in the diets. On the basis of the results from study I and II, the EFSA GMO Panel considers it unlikely that the observed differences in animal body-weights in both studies are related to the exposure to processed meal of soybean MON 87701.

There were no treatment-related deaths in any of the two studies, and no relevant differences in clinical findings between test and control groups. Regarding haematology, coagulation parameters and serum chemistry, statistically significant differences between test and control groups were observed. Some of the differences only occurred in rats administered 15 % MON 87701 soybean meal in the diet of study II and were thus not considered treatment related. The differences found in animals receiving diets containing 30 % MON 87701 soybean meal (i.e. , lower mean haemoglobin level and eosinophil counts in female animals in study II; lower mean total protein and higher chloride levels in female animals in study I; lower mean phosphorus levels in males in study II) are regarded by the EFSA GMO Panel as incidental since the values were within the historical control ranges and there were no changes in related parameters which could indicate a specific organ toxicity. Furthermore, the differences were only observed in one of the two studies and not reproduced in the other one. In both studies, urinalysis did not show toxicologically relevant differences between groups.

Macroscopic examinations at necropsy did not reveal relevant changes in both studies. Regarding organ weights in study I, male rats given the MON 87701-diet showed reduced absolute spleen weights, and altered spleen weight/final body weight and spleen weight/brain weight ratios as compared to male rats in the control group. However, the values for these parameters in the MON 87701 soybean meal-exposed animals were comparable to the values for these parameters in male rats receiving the reference diets and in historical controls. Furthermore, there were no histopathological findings in the microscopic examination of this organ. The differences in spleen weight parameters observed in males are therefore not considered to be related to the exposure to processed MON 87701 soybean meal. The statistically significant differences in absolute organ weights observed in females (i.e. lower brain, kidney, liver and spleen weights) are considered to be a consequence of the lower body weight and lower body weight gain during the study (see above) since the respective organ weights relative to body weights were not affected. Furthermore, there were no histological alterations in these organs which would indicate adverse effects.

Study II showed several statistically significant differences between animals that had received 15 % MON 87701 soybean meal in the diet and animals of the control group (15 % processed soybean meal of A5547). Such differences (kidney, liver and thyroid/parathyroid weight in males, adrenal gland weight relative to brain weight in females) were not observed in the high-dose group (30 % soybean MON 87701 meal), and are thus considered as incidental. The applicant considered these observations to be a consequence of the slightly higher body weights in males given 15 % MON 87701 in the diet. In the high dose group, statistically significantly lower epididymide and teste weights (both relative to final body weight) were noted in male rats, and higher mean thyroid/parathyroid weights (absolute and relative to final body weight and brain weight) in female rats. The differences were small, and the group means of these parameters were within the range in group means of the historical controls receiving 30 % processed soybean meal. Since no differences for these parameters were found in study I and no histopathological alterations were observed in that study, and there were also no clinical or gross pathological findings in the repeated study, the observed differences in epididymide, teste and thyroid/parathyroid weights in study II are not considered related to the exposure to MON 87701 soybeans. Microscopic examinations of other organs and tissues carried out in study I revealed no relevant differences between the test and control group (no microscopic examinations were carried out in study II).

The EFSA GMO Panel concludes that administration of diets containing 30 % processed meal of soybean MON 87701 to rats did not cause adverse effects.

5.1.4. Allergenicity[19]

The strategies used when assessing the potential allergenic risk focus on the characterisation of the source of the recombinant protein, the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons and whether the transformation may have altered the allergenic properties of the modified 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; EFSA Panel on Genetically Modified Organisms (GMO), 2010).

5.1.4.1. Assessment of allergenicity of the newly expressed proteins

The *cry1Ac* gene originates from *B. thuringiensis* subsp. *kurstaki*, a soil-borne and plant-interacting micro-organism that is not known to be allergenic. The Cry1Ac protein is expressed in various tissues of soybean MON 87701, except roots, and has been quantified at a number of growth stages during the growing season (see section 3.1.3.). The most relevant tissue for the assessment of food allergenicity is the seed, which contains around 5 µg Cry1Ac/g dry weight (i.e. around 0.0013 % of total soybean protein).

A bioinformatics-supported comparison of the amino acid sequence of the Cry1Ac protein with the sequences of known allergens, gliadins, and glutenins, collected in an updated proprietary database based on the FARRP database, was performed. This analysis included both overall sequence alignments using the FASTA algorithm and searches for short identical stretches of at least eight contiguous amino acids. In the overall sequence alignment, no identity higher than 35 % in polypeptides of 80 or more amino acids was found between the Cry1Ac protein and known allergens. Similarly, no identical sequence of eight contiguous amino acids was detected. As described above, Cry1Ac is degraded under simulated gastric and intestinal conditions. Based on these results, the GMO Panel considers that the newly expressed Cry1Ac protein is unlikely to be allergenic in the intended conditions of exposure.

5.1.4.2. Assessment of allergenicity of the whole GM plant or crop

Allergenicity of the whole crop 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 allergens. However, given that the comparative compositional and agronomical analysis revealed soybean MON 87701 not to be different from the conventional counterpart except for soybean MON 87701 expressing the Cry1Ac protein and showing increased levels of vitamin E, no increased allergenicity is anticipated for soybean MON 87701. Because soybean is a recognised allergenic food, the applicant performed extensive *in vitro* allergenicity studies with extracts of soybeans MON 87701, A5547 (the conventional counterpart of soybean MON 87701), and 17 different commercial soybean varieties. The IgE-binding of soybean proteins to sera from 13 individuals clinically documented allergic to soybean, and 5 non-allergic individuals were quantified with an ELISA method in order to demonstrate that the allergenicity potential of soybean MON 87701 is not altered in comparison to conventional soybean varieties. Whereas proteins from none of the soybean varieties showed binding to sera from non-allergic individuals, most serum samples from allergic individuals had similar reactivity to proteins in extracts from soybean MON 87701 and soybean A5547. The applicant

[19] Technical dossier/section D7.9

supplied two-dimensional (2D) electrophoresis of water extracts of soybean MON 87701 and A5547 followed by Western blotting with human IgE antibodies from allergic individuals to further address the potential for changes in endogenous allergenicity of soybean 87701. These studies demonstrated no meaningful qualitative and quantitative difference in the IgE-binding patterns to proteins of extracts derived from soybean MON 87701 and soybean A5547 for all the sera studied except for the serum of one very high IgE-responder allergic individual. The differences observed in the *in vitro* studies with this particular allergic individual may be due to the heterogeneity of the human IgE response of the sera samples and differences in the endogenous protein expression in the crop and do not raise concern under the intended conditions of use. Therefore, the EFSA GMO Panel is of the opinion that these studies do not indicate a consistent and biologically significant modification of the overall allergenicity of soybean MON 87701 as compared to that of its conventional counterpart.

5.1.5. Nutritional assessment of GM food/feed[20]

As the molecular characterisation of soybean MON 87701 identified no unintended effects of the genetic modification, the newly expressed Cry1Ac protein was found safe for higher animals, and the comparative compositional and agronomical analysis revealed soybean MON 87701 to be equivalent to commercial soybean varieties except for soybean MON 87701 expressing the Cry1Ac protein, a nutritional equivalence to conventional soybeans can be assumed. According to the 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), long-term livestock feeding studies with the whole food/feed are not required in these cases.

The applicant provided a 42-day broiler chicken feeding study (Cobb × Cobb 500) with defatted soybean meal performed according to generally accepted guidelines (ILSI, 2003). The study consisted of nine treatment groups: one group received soybean MON 87701, another group received soybean MON 87701 × MON 89788 (not included in the analysis for soybean MON 87701), another group received soybean A5547 (conventional counterpart of MON 87701), and the other six groups different conventional non-GM soybean varieties.

Each treatment consisted of 60 male and 60 female broilers allocated in pens of 12 chickens per pen, that were reduced to 10 birds/pen at day 7. A randomised complete block design with 5 blocks of 18 pens was used. Animals were fed adjusted diets containing approximately 33 % (w/w) of soybean meal in the starter diet (day 0-21) and 30 % soybean meal in the grower/finisher diet (day 21-42). The diets were formulated based on nutrient requirements recommended by the National Research Council (NRC, 1994), and were quality controlled, including confirmation that levels of pesticides and mycotoxins were below threshold levels of concern for feeding studies.

The different treatment groups showed a chicken mortality between 0.8 % and 5.0 % during the first 7 days of the study, mainly due to bacterial infection and dehydration, and between 0.0 % and 5.0 % during day 7-42 of the study, being most deaths due to ascites or sudden death syndrome. A total mortality rate of 10 % for the soybean MON 87701-fed chickens may be incidental but is not considered good practice by the EFSA GMO Panel for scientific studies devoted to nutritional wholesomeness/safety testing.[21] However, feeding of broiler chickens with products of soybean MON 87701 had no effects on feed intake, final body weight (around 2.5 kg per animal), and weight gain of broilers. Only adjusted feed conversion rates differed slightly between the treatment groups, ranging from 1.52 to 1.56 kg feed per kg weight gain for the various groups. The lowest value was obtained from broiler chickens fed soybean MON 87701 (but only in females). No difference was observed in the various parameters of carcass yield, neither in fat, protein and moisture content of breast and thigh meat. However, there was a diet-sex interaction for three of the parameters tested: percent fat pad

[20] Technical dossier/section D7.10
[21] Additional information July 2010

weight and thigh weight were slightly reduced in males, and percent chilled weight was slightly reduced in females.[22]

In conclusion, the broiler feeding study identified no relevant difference in broiler performance, carcass yield or meat composition between chickens fed diets containing extracted soybean meal produced from soybean MON 87701 and the conventional counterpart or other conventional soybean varieties. The data confirm the results of the comparative compositional analysis that indicated that soybean MON 87701 is compositionally and, therefore, implicitly as nutritious as commercial soybean varieties, including the conventional counterpart.

5.1.6. Post-market monitoring of GM food/feed

The risk assessment concluded that no data have emerged to indicate that soybean MON 87701 is less safe than its conventional counterpart. In addition, soybean MON 87701 is as nutritious as conventional soybean. Therefore, and in line with the 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), the EFSA GMO Panel is of the opinion that post-market monitoring of the GM food/feed is not necessary.

5.2. Conclusion

The Cry1Ac is degraded in simulated digestive and intestinal fluids, and bioinformatics-supported studies demonstrated that the Cry1Ac protein show no homology to known toxic and allergenic proteins. No toxicity of the Cry1Ac protein was observed in an acute toxicity study in mice where the protein was administered orally at a high dose. The result of 90-day feeding studies with toasted defatted soybean meal from MON 87701 in rats did not raise concern. Whole-product testing of soybean extracts to sera from soy-allergic patients showed that the overall allergenicity of the whole plant had not been changed. A 42-day feeding study on broiler chickens showed that soybean MON 87701 is as nutritious as conventional counterpart and other soybean varieties included in the study. In conclusion, the EFSA GMO Panel is of the opinion that soybean MON 87701 is as safe and as nutritious as its conventional counterpart and commercial soybean varieties in the context of its intended use.

6. Environmental risk assessment and monitoring plan

6.1. Environmental risk assessment

The scope of this application EFSA-GMO-BE-2010-79 is for food and feed uses, import and processing and does not include cultivation. Considering the intended uses of soybean, the environmental risk assessment is concerned with the indirect exposure mainly from manure and faeces from animals fed with soybean MON 87701 and with the accidental release into the environment of viable grains of soybean MON 87701 during transportation and processing.

Soybean MON 87701 has been developed for protection against certain lepidopteran pests (i.e. *A. gemmatalis, P. includens, E. aporem* and *R. nu* which are not present in European fauna). Insect resistance is achieved by the expression of the *B. thuringiensis* derived Cry1Ac protein.

[22] Additional information December 2010

6.1.1. Unintended effects on plant fitness due to the genetic modification[23]

Cultivated soybean (*Glycine max* (L.) Merr.) is a species in the subgenus *Soja* of the genus *Glycine*. The species originated from eastern Asia and is a highly domesticated crop (Lu, 2005). The major worldwide soybean producers are the United States (US), Brazil, Argentina, China, North Korea and South Korea. In the European Union , soybean is mainly cultivated in Austria, Italy, France, Hungary and Romania (Dorokhov et al., 2004).[24]

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 due to predation, rotting, germination resulting in death, or due to management practices prior to planting the subsequent crop (Owen, 2005).

Field trials with soybean MON 87701 were carried out by the applicant across 16 locations in the US in 2007 and 8 locations in Argentina in 2007/2008 as described in section 4.1.3. As mentioned above, no statistically significant difference was observed in the combined site analysis of the field trials data of the 2007 season. The combined site analysis of the 2007/2008 field data identified three statistically significant differences in early stand count, seed moisture and test weight of harvested seeds.

The EFSA GMO Panel considers that the differences observed in early stand count, seed moisture, and weight of harvested grains are unlikely to affect the overall fitness and weed potential of the GM soybean, except under infestation conditions of specific target organisms.

Seed germination and dormancy characteristics, pollen morphology and viability were evaluated as described in section 4.1.3.

These field trial and laboratory data do not show altered agronomic performance that would indicate any change in fitness and invasiveness or weediness of soybean MON 87701 compared to conventional soybean varieties, except under infestation conditions of specific target organisms.

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 survival capacity, including overwintering (Dorokhov et al., 2004, Owen, 2005, Bagavathiannan and Van Acker, 2008, Lee et al., 2009).

Survival of soybean plants outside cultivation areas is mainly limited by a combination of low competitiveness, absence of a dormancy phase, and susceptibility to plant pathogens and cold climate conditions. Since these general characteristics are unchanged in soybean MON 87701, it can be considered that soybean MON 87701 has no altered survival, multiplication or dissemination characteristics, except under infestation conditions by specific target pests. Therefore, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects of the soybean MON 87701 in Europe will not be different to that of conventional soybean varieties.

6.1.2. Potential for gene transfer[25]

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

[23] Technical dossier/sections D4, D9.1 and D9.2
[24] http://epp.eurostat.ec.europa.eu/portal/page/portal/agriculture/data/database
[25] Technical dossier/section D6

(a) 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 present in the digestive tract of humans, domesticated animals, and other animals feeding on soybean MON 87701 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 to 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 87701 contains genetic elements with identity or high similarity to those of bacteria. The coding sequence of Cry1Ac is a synthetic gene which is highly similar to corresponding genes from Cry1Ac producing *B. thuringiensis* and the flanking regions of the recombinant gene insert contain approximately 50 and 260 bp long sequences of the right and left border of the Ti-plasmid of *A. tumefaciens*, respectively. Both species, *A. tumefaciens* and *B. thuringiensis*, are not considered to be prevalent in the main receiving environment, i.e. the gastrointestinal tract of humans or animals. Both occur in soil, and in addition, *B. thuringiensis* has been frequently isolated from guts of insects (Jensen et al., 2003).

On a theoretical basis (i.e. without any study providing experimental evidence for horizontal gene transfer (HGT) in the case of GM food and feed derived from soybean MON 87701 or any other GM plant) it can be assumed that, as an extremely rare event, homologous recombination may occur between the recombinant *cry1Ac* gene and the *cry1Ac* gene of *B. thuringiensis* present in the environment. Such substitutive recombination events are unlikely to provide 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 loose its capability of crown gall formation (loss of auxin, cytokinin and opine synthesizing genes).

In addition to homology-based recombination processes, illegitimate recombination that does not require the presence of DNA similarity between the recombining DNA molecules is theoretically possible. However, the transformation rates for illegitimate recombination were considered to be 10^{10}-fold lower than for homologous recombination (Hülter and Wackernagel, 2008; EFSA, 2009). Illegitimate recombination events have not been detected in studies that have exposed bacteria to high concentrations of GM-plant DNA (EFSA, 2009). Thus this process in comparison to HR is not considered to significantly contribute to horizontal gene transfer events. In comparison to the above described homology-facilitated recombination processes, the contribution of illegitimate recombination is extremely low.

The *cry1Ac* gene of soybean MON 87701 is regulated by a eukaryotic plant promoter (derived from the *A. thaliana RbcS4*). The expression level of eukaryotic promoters in bacteria is variable, but often

inefficient (Warren et al., 2008). The expression of the *prRBCS4-cry1ac* construct in bacteria is unknown.

In a worst case scenario, considering the possibility of expression, an *A. tumefaciens* recipient would become capable of producing an insecticidal Cry1Ac protein. The exposure of bacterial communities to the recombinant gene in soybean MON 87701 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. Due to its specific life-style as a soil bacterium and plant pathogen, in contrast to the life-style of *B. thuringiensis*, which colonizes insect guts and infects specific target insects, the EFSA GMO Panel considers unlikely that *A. tumefaciens* would gain selective advantage from such a HGT by double homologous recombination.

The EFSA GMO Panel concludes that the *cry1Ac* gene from soybean MON 87701 may, on a theoretical basis, be transferred by double homologous recombination to *A. tumefaciens* or to *B. thuringiensis*. However, since both *A. tumefaciens* and *B. thuringiensis* are not considered to be members of the gut microbiota, exposure to recombinant DNA of MON 87701 is considered to be very low. Due to the natural occurrence of *cry1Ac* in the environment, a low level gene transfer to *A. tumefaciens* or gene replacement in *B. thuringiensis* is not regarded to confer a novel selective advantage. Considering its intended use as food and feed and the above assessment, the EFSA GMO Panel has therefore not identified a concern associated with a HGT from MON 87701 to bacteria.

(b) Plant to plant gene transfer

Considering the intended uses of soybean MON 87701 and the physical characteristics of soybean seeds, a possible pathway of gene dispersal is from seed spillage and pollen of occasional feral GM soybean plants originating from accidental seed spillage mainly during transportation 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, whilst the cultivated soybean, *G. max*, and its wild and semi-wild annual relatives, *Glycine soja* and *Glycine gracilis*, are classified in the subgenus *Soja* (OECD, 2000). Due to the low level of genomic similarity among species of the genus *Glycine*, *G. max* can only cross 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 *G. soja* and *G. gracilis* are indigenous to China, Taiwan, Korea, Japan, the Far East Region of Russia, Australia, the Philippines and 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 of some within-crop gene flow. 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 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 87701 seeds (while most soybean MON 87701 seeds will be processed in countries of production), processing outside of importing ports, transportation in regions of soybean production in Europe, spillage of GM seeds mainly during transportation, germination and development of spilled seeds within soybean fields or in very close vicinity of cultivated soybean fields, overlap of flowering periods and environmental conditions favouring cross-pollination. The likelihood of all these conditions occurring and thereby resulting in 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 overtime. Dispersal of soybean seeds by animals is not expected due to the characteristics of the seed, but accidental release into the environment of seeds may occur (e.g. during transportation and processing for food, feed and industrial uses). However, cultivated soybean seeds rarely display any dormancy characteristics and grow only under certain environmental conditions as volunteers in the year following cultivation (OECD, 2000). Even in soybean fields, seeds usually do not survive during the winter due to predation, rotting, germination resulting in death, or due to 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.

In conclusion, since soybean MON 87701 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 soybean MON 87701 in Europe will not differ from that of conventional soybean varieties.

6.1.3. Interactions of the GM plant with target organisms[26]

Due to the intended uses of soybean MON 87701, which exclude cultivation, and due to the low level of exposure to the environment, potential interactions of the GM plant with target organisms were not considered an issue by the EFSA GMO Panel.

6.1.4. Interactions of the GM plant with non-target organisms[27]

Due to the intended uses of soybean MON 87701, which exclude cultivation, and due to 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.

However, the EFSA GMO Panel evaluated whether the Cry1Ac protein might potentially affect non-target organisms by entering the environment through manure and faeces from animals fed this GM soybean. Cry proteins are degraded by enzymatic activity in the gastrointestinal tract, meaning that only a very low amount of these proteins would remain intact to pass out in faeces. This was demonstrated for Cry1Ab (Einspanier et al., 2004; Lutz et al., 2005; Lutz et al., 2006; Wiedemann et al., 2006; Guertler et al., 2008; Paul et al., 2010). There would, subsequently, be further degradation of the protein in the manure and faeces due to microbiological proteolytic activity.

In addition, there will be further degradation of Cry proteins in soil reducing the possibility for exposure of potentially sensitive non-target organisms. While Cry proteins may bind to clay minerals

[26] Technical dossier/sections D8 and D9.4
[27] Technical dossier/section D9.5

and humic substances in soil, thereby reducing their availability to micro-organisms for degradation, there are no indications of persistence and accumulation of Cry proteins from GM crops in soil (reviewed by Icoz and Stotzky, 2008). The EFSA GMO Panel is not aware of evidence of released Bt toxins protein causing significant negative effects on soil micro-organisms.

Considering the scope of the application, it can be concluded that the exposure of potentially sensitive non-target organisms to the Cry1Ac protein is likely to be very low and of no biological relevance.

6.1.5. Interactions with the abiotic environment and biogeochemical cycles[28]

Due to the intended uses of soybean MON 87701, which exclude cultivation, and due to the low level of exposure to the environment, potential interaction of the GM plant with the abiotic environment and biogeochemical cycles were not considered an issue by the EFSA GMO Panel.

6.2. Post-market environmental monitoring[29]

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 which were not anticipated in the environmental risk assessment.

Monitoring is 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). The potential exposure to the environment of soybean MON 87701 would be through manure and faeces from animals fed soybean MON 87701 or through accidental release into the environment of GM soybean seeds (e.g. during transportation and processing). The EFSA GMO Panel is aware that, due to 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) and the scientific opinion of the EFSA GMO Panel on post-market environmental monitoring (EFSA, 2006b).

The scope of the monitoring plan provided by the applicant is in line with the intended uses. Since 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 applicant via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment, (2) a coordinating system established by EuropaBio for the collection of the information recorded by the various operators, and (3) the use of networks of existing surveillance systems (Lecoq et al., 2007; Windels et al., 2008). The applicant proposes to submit a general surveillance report on an annual basis and a final report at the end of the consent.

The EFSA GMO Panel is of the opinion that the scope of the monitoring plan proposed by the applicant is in line with the intended uses of soybean MON 87701 since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. The

[28] Technical dossier/sections D9.8 and D10
[29] Technical dossier/section D11

EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

6.3. Conclusion

The scope of the application ifs for food and feed uses, import and processing of soybean MON 87701 and excludes cultivation. Considering the intended uses, the environmental risk assessment is concerned with indirect exposure mainly through manure and faeces from animals fed seeds produced by soybean MON 87701 and with the accidental release into the environment of viable seeds of soybean MON 87701 (e.g. during transportation and processing).

In case of accidental release into the environment of viable seeds of soybean MON 87701 (e.g. during transportation and processing), there are no indications of an increased likelihood of establishment and spread of feral soybean MON 87701 plants, except under infestation conditions of specific target organisms. 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 unlikely but theoretically possible transfer of the recombinant gene from soybean MON 87701 to environmental bacteria does not raise concern due 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 87701.

The EFSA GMO Panel is aware that, due to physical characteristics of soybean seed and methods of transportation, accidental spillage cannot be excluded. Therefore, the EFSA GMO Panel recommends that, within general surveillance, appropriate management systems are introduced to actively monitor the occurrence of feral soybean plants in areas where spillage and soybean plant establishment are likely to occur as proposed in the 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) and the scientific opinion of the EFSA GMO Panel on post-market environmental monitoring (EFSA, 2006b).

The EFSA GMO Panel also recommends that appropriate management systems should be in place to restrict seeds of soybean MON 87701 entering cultivation as this would require specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

CONCLUSIONS AND RECOMMENDATIONS

The EFSA GMO Panel was requested to carry out an evaluation of a scientific risk assessment of the soybean MON 87701 for food and feed uses, import and processing in accordance with Regulation (EC) No 1829/2003.

The EFSA GMO Panel is of the opinion that the molecular characterisation data provided for soybean MON 87701 are sufficient to conclude on this part of the risk assessment evaluation. The results of the bioinformatic analyses of the inserted DNA and the flanking regions do not raise safety concerns. The levels of Cry1Ac protein in soybean MON 87701 have been sufficiently analysed in various tissues and the stability of the genetic modification has been demonstrated. The EFSA GMO Panel considers that the molecular characterisation does not indicate a safety concern.

Based on the comparative analysis of soybean MON 87701, the conventional counterpart (A5547) and several other commercial soybean varieties, the EFSA GMO Panel concludes that soybean MON 87701, as assessed in this application, is compositionally, phenotypically and agronomically not different from its conventional counterpart except for having an increased vitamin E content (still within the normal range of soybeans) and expressing the Cry1Ac protein. Except for expressing the Cry1Ac protein, soybean MON 87701 is also compositionally and agronomically equivalent to commercial soybean varieties.

The Cry1Ac protein expressed in MON 87701 is degraded in simulated digestive and intestinal fluids, and bioinformatics-supported studies demonstrated that the Cry1Ac protein show no homology to known toxic and allergenic proteins. No toxicity of the Cry1Ac protein was observed in an acute toxicity study in mice where the protein was administered orally at a high dose.

The result of 90-days feeding studies in rats with processed soybean MON 87701 meal did not raise concern. Whole-product testing of soybean extracts to sera from soy-allergic patients demonstrated unchanged overall allergenicity of the whole plant. A 42-day feeding study on broiler chickens showed that defatted soybean meal from MON 87701 is as nutritious as meal from the conventional counterpart and other soybean varieties included in the study.

Considering the intended uses of soybean MON 87701, which exclude cultivation, there is no requirement for scientific assessment on possible environmental effects associated with the cultivation of this GM soybean. In case of accidental release into the environment of viable seeds of soybean MON 87701 (e.g.; during transportation and processing), there are no indications of an increased likelihood of establishment and spread of feral soybean plants, except under infestation conditions of specific target organisms. 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 unlikely but theoretically possible transfer of the recombinant gene from soybean MON 87701 to environmental bacteria does not raise concern due 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 87701. The EFSA GMO Panel is aware that, due to physical characteristics of soybean seed and methods of transportation, accidental spillage cannot be excluded. Therefore, the EFSA GMO Panel recommends that, within general surveillance, 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.

In conclusion, the EFSA GMO Panel considers that the information available for soybean MON 87701 addresses the scientific comments raised by the Member States and that the soybean MON 87701, as described in this 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.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of Belgium, received 17 May 2010, concerning a request for placing on the market of Soybean MON 87701 submitted by Monsanto under Regulation (EC) No 1829/2003.

2. Acknowledgement letter, dated 4 June 2010, from EFSA to the Competent Authority of Belgium (Ref. CGL/RM/PB/KL/lg (2010) 4896301).

3. Letter from EFSA to applicant, dated 11 June 2010, delivering the 'Statement of Validity' for application EFSA-GMO-BE-2010-79, Soybean MON 87701 submitted by Monsanto under Regulation (EC) No 1829/2003 (Ref. PB/KL/CE/mt (2010) 4923433).

4. Letter from EFSA to applicant, dated 21 June 2010, requesting additional information and stopping the clock (Ref. PB/KL/JA/shv (2010) 4937036).

5. Letter from applicant to EFSA, received 1 July 2010, providing additional information.

6. Letter from EFSA to applicant, dated 12 November 2010, requesting additional information and maintaining the clock stopped (Ref. PB/KL/JA/mt (2010) 5312865).

7. Letter from applicant to EFSA, received 3 January 2011, providing additional information.

8. Letter from EFSA to applicant, dated 4 March 2011, requesting additional information and maintaining the clock stopped (Ref. PB/KL/JA/lg (2011) 5610867).

9. Letter from applicant to EFSA, received 15 March 2011, providing additional information.

10. Letter from EFSA to applicant, dated 31 May 2011, restarting the clock (Ref. PB/KL/JA/mt (2011) 5803594).

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