SCIENTIFIC OPINION

Scientific opinion on application (EFSA-GMO-NL-2009-73) for the placing on the market of insect-resistant and herbicide-tolerant genetically modified soybean MON 87701 × MON 89788 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 for placing on the market the genetically modified (GM) insect-resistant and herbicide-tolerant soybean MON 87701 × MON 89788 for food and feed uses, import and processing. Soybean MON 87701 × MON 89788 was produced by conventional crossing methods, and the F_1 plant is hemizygous for all newly introduced traits. The soybean contains the *Cry1Ac* and CP4 *epsps* genes conferring resistance against certain lepidopteran target pests and tolerance to glyphosate-based herbicides. No biologically relevant differences were identified in the composition or agronomic and phenotypic characteristics of soybean MON 87701 × MON 89788, as compared with its comparator, except that it expresses the Cry1Ac and CP4 EPSPS proteins. The safety assessment identified no concerns regarding the potential toxicity and allergenicity of soybean MON 87701 × MON 89788. 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 with an unlikely but theoretically possible horizontal gene transfer from soybean MON 87701 × MON 89788 to bacteria have not been identified. Potential interactions of soybean MON 87701 × MON 89788 with the biotic and abiotic environment were not considered to be an issue owing to the low level of exposure. The monitoring plan and reporting intervals are in line with the intended uses of soybean MON 87701 × MON 89788. In conclusion, the EFSA GMO Panel considers that the information available for soybean MON 87701 × MON 89788 addresses the scientific comments raised by Member States and that the soybean MON 87701 × MON 89788, as described in this application, is as safe as its comparator with respect to potential effects on human and animal health and the environment, in the context of its intended uses.

[1] On request from the Competent Authority of the Netherlands for an application (EFSA-GMO-NL-2009-73) submitted by Monsanto, Question No EFSA-Q-2009-00761, adopted on 26 January 2012.

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KEY WORDS

GMO, soybean (*Glycine max*), MON 87701 × MON 89788, insect-resistant, herbicide-tolerant, human and animal health, import and processing, Regulation (EC) No 1829/2003.

SUMMARY

Following the submission of an application (EFSA-GMO-NL-2009-73) under Regulation (EC) No 1829/2003[4] 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 × MON 89788 (Unique Identifier MON-877Ø1-2 × MON-89788-1) for food and feed uses, import and processing.

In delivering its scientific opinion, the EFSA GMO Panel considered the application EFSA-GMO-NL-2009-73, additional information supplied by the applicant, scientific comments submitted by the Member States and relevant scientific publications. Further information from applications for placing on the market under European Union regulatory procedures the single soybean events MON 87701 and MON 89788 was taken into account. The scope of application EFSA-GMO-NL-2009-73 is for food and feed uses, import and processing of soybean MON 87701 × MON 89788 within the EU in the same way as any non-GM soybean but excludes cultivation in the EU. The EFSA GMO Panel evaluated soybean MON 87701 × MON 89788 with reference to the intended uses and appropriate principles described in its guidance documents of the Scientific Panel on Genetically Modified Organismsfor the risk assessment of genetically modified plants and derived food and feed (EFSA 2006) and for the risk assessment of GM plants containing stacked transformation events (EFSA 2007). 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 the composition and 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 was also undertaken.

The single soybean events MON 87701 and MON 89788 were the subject of separate earlier risk assessment evaluations by the EFSA GMO Panel. The EFSA GMO Panel concluded that they are unlikely to have any adverse effect on human and animal health and the environment, in the context of their intended uses (EFSA 2008, 2011a). The placing on the market of products containing, consisting of or produced from genetically modified soybean MON 89788 was authorised pursuant to Regulation (EC) No 1829/2003.[5] No new genes, in addition to those occurring in soybean MON 87701 and MON 89788, have been introduced in soybean MON 87701 × MON 89788. Soybean MON 87701 × MON 89788 was produced by conventional crossing of the single soybean events to combine in the same stack resistance against certain lepidopteran target pests and tolerance to glyphosate-based herbicides.

Molecular analysis has confirmed that soybean MON 87701 and MON 89788 inserts are present and that their structures are retained in soybean MON 87701 × MON 89788. The result of the updated bioinformatic analyses of the flanking sequences and the open reading frames spanning the insert–plant DNA junctions did not reveal a safety concern. The overall levels of the Cry1Ac and CP4 EPSPS proteins were comparable to those of the corresponding single soybean events MON 87701 and MON 89788.

The EFSA GMO Panel compared the composition and phenotypic and agronomic characteristics of soybean MON 87701 × MON 89788 with its comparator (A5547), assessed all statistically significant differences identified, and came to the conclusion that no biologically relevant differences were identified in the composition or phenotypic and agronomic characteristics of soybean MON 87701 × MON 89788 as compared with its comparator (A5547) and that the composition fell within the range of non-GM soybean varieties, except that soybean MON 87701 × MON 89788 expressed the CP4 EPSPS and Cry1Ac proteins. A small increase in final stand count in soybean MON 87701 × MON 89788 was observed, but no safety issues were identified linked to this increase. The risk assessment included an analysis of data from analytical and bioinformatics studies, as well

[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] Commission decision of 4 December 2008 authorising the placing on the market of products containing, consisting of or produced from genetically modified soybean MON 89788 (MON-89788-1) pursuant to Regulation (EC) No 1829/2003 of the European Parliament and of the Council. Official Journal of the European Union L 333/7–10.

as in vitro and in vivo studies. The EFSA GMO Panel concluded that soybean MON 87701 × MON 89788 is as safe as its comparator and that the overall allergenicity of the whole plant has not changed.

Potential interaction between the soybean events with respect to an effect on human and animal health were the focus of the assessment on food/feed issues. On the basis of the known functional characteristics and modes of action of the newly expressed proteins (Cry1Ac and CP4 EPSPS), the EFSA GMO Panel considers it unlikely that interactions between these proteins would occur that would raise any safety concerns. Thus, the Panel is of the opinion that soybean MON 87701 × MON 89788 is as safe and as nutritious as its comparator and commercial soybean varieties, in the context of its intended uses.

The application EFSA-GMO-NL-2009-73 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 × MON 89788. There are no indications of an increased likelihood of the establishment and spread of feral soybean plants in the event of the accidental release into the environment of viable soybean MON 87701 × MON 89788 grains during transport and processing for food and feed uses, except under conditions of infestation by the specific lepidopteran pests or the application of glyphosate-based herbicides. Taking into account the scope of the application, both the rare occurrence of feral soybean plants and the low levels of exposure to the environment 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 × MON 89788 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 is in line with the intended uses of soybean MON 87701 × MON 89788. Furthermore, the EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in its general surveillance plan.

In conclusion, the EFSA GMO Panel considers that the information available for soybean MON 87701 × MON 89788 addresses the scientific issues indicated by the guidance document of the EFSA GMO Panel and the scientific comments raised by the Member States, and that soybean MON 87701 × MON 89788 is as safe as its comparator with respect to potential effects on human and animal health or the environment in the context of its intended uses. In addition, the EFSA GMO Panel is of the opinion that crossing of single soybean events MON 87701 and MON 89788 to produce soybean MON 87701 × MON 89788 does not result in interactions between the events that would affect the safety of soybean MON 87701 × MON 89788 with respect to potential effects on human and animal health and on the environment, in the context of its intended uses.

TABLE OF CONTENTS

BACKGROUND

On 27 August 2009, EFSA received from the Dutch Competent Authority an application (Reference EFSA-GMO-NL-2009-73) for authorisation of genetically modified (GM) soybean MON 87701 × MON 89788 (Unique Identifier MON-877Ø1-2 × MON-89788-1) submitted by Monsanto within the framework of Regulation (EC) No 1829/2003 on GM food and feed. After receiving the application EFSA-GMO-NL-2009-73, 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 8 December 2009, 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 EC and consulted nominated risk assessment bodies of the Member States, including the national Competent Authorities within the meaning of Directive 2001/18/EC[6], following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Member States had three months after the date of receipt of the valid application (until 8 March 2010) within which to make their opinion known.

The EFSA Scientific Panel on Genetically Modified Organisms (EFSA GMO Panel) carried out an evaluation of the scientific risk assessment of the GM soybean MON 87701 × MON 89788 for food and feed uses, import and processing, in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. The EFSA GMO Panel 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 2006). In addition, the scientific comments of the Member States, additional information provided by the applicant and relevant scientific publications were taken into consideration.

On 26 February 2010, 8 July 2010, 26 August 2011, 13 October 2011 and 2 December 2011, the EFSA GMO Panel requested additional information from the applicant. The applicant provided the requested information on 9 April 2010, 5 September 2011, 3 November 2011 and 6 December 2011.

In giving its opinion on soybean MON 87701 × MON 89788 to the EC, the Member States and the applicant, and in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003, EFSA has endeavoured to respect a time limit of 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 × MON 89788 for food and feed uses, import and processing, in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. Where applicable, any conditions or restrictions that should be imposed on its 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

[6] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Official Journal of the European Communities, L106, 1–38.

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 × MON 89788 (Unique Identifier MON-877Ø1-2 × MON-89788-1) was evaluated with reference to its intended uses, taking account of the appropriate principles described in the guidance documents of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (EFSA 2006) and for the risk assessment of GM plants containing stacked transformation events (EFSA 2007). 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[7] and have been considered in this scientific opinion.

3. MOLECULAR CHARACTERISATION

3.1. Evaluation of relevant scientific data

3.1.1. Method of production of soybean MON 87701 × MON 89788

Conventional breeding methods were used to develop soybean MON 87701 × MON 89788, and no new genetic modification was involved[8]. The two inserts that are present in soybean MON 87701 × MON 89788 were derived from soybean lines containing two independent events: MON 87701 and MON 89788. Genetically modified soybeans MON 89788 and MON 87701 were the subjects of earlier safety evaluations (EFSA 2008, 2011a). Soybean MON 87701 × MON 89788 combines resistance to certain lepidopteran pests with tolerance to glyphosate-based herbicide.

3.1.2. Summary of the evaluation of the single events

3.1.2.1. MON 87701

Soybean MON 87701 was developed through *Agrobacterium*-mediated transformation and, as a result, expresses the *cry1Ac* gene, under the control of *Arabidopsis thaliana rbcS4* promoter, to confer resistance to specific lepidopteran insects. Molecular characterisation data have established that MON 87701 contains a single insert with one copy of the intact Cry1Ac expression cassette at a single locus and that vector backbone sequences are absent. A comparison with the pre-insertion locus of the parental soybean A5547 indicated that a 32-bp fragment of endogenous DNA has been deleted in soybean MON 87701, and 14 bp have been introduced immediately 5′ to the insertion site. The results of bioinformatic analysis did not indicate the interruption of a soybean coding sequence(s) with known function in the MON 87701 event. The analysis of cryptic open reading frames (ORFs) in the MON 87701 event did not indicate any alignment that would meet or exceed the Codex Alimentarius (2009) threshold for potential allergenicity, and no relevant similarities to known toxic proteins other than Bt proteins (Cry1Ac) were found. The *cry1Ac* gene was shown to be stably inherited and the inheritance followed a Mendelian segregation pattern. A more detailed evaluation of the MON 87701 event can be found in a previous EFSA opinion (EFSA 2011a).

3.1.2.2. MON 89788

Soybean MON 89788 was developed through *Agrobacterium*-mediated transformation and, as a result, expresses the CP4 *epsps* gene, conferring tolerance to glyphosate-based herbicides, under the control of the chimeric promoter consisting of enhancer sequences from the 35S promoter of the figwort mosaic virus and the promoter from the *Tsf1* gene of *Arabidopsis thaliana*. Molecular characterisation data established that MON 89788

[7] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2009-00761
[8] Technical Dossier/Section C

contains a single insert with one copy of the intact CP4 *epsps* expression cassette at a single locus and that vector backbone sequences are absent. Similarity searches revealed that the flanking regions of the insert in soybean MON 89788 show significant level of identity to soybean genomic DNA sequences and indicated that the pre-insertion locus was preserved except for the deletion of 40 bp. Bioinformatic analysis confirmed that no known endogenous soybean ORFs or regulatory sequences have been disrupted by the insert. The bioinformatic analysis revealed no biologically relevant similarities to allergens or toxins for any of the putative (poly)peptides that might be produced from ORFs spanning the junction regions. Southern analysis of MON 89788 and maintenance of the phenotype indicated genetic and phenotypic stability of the event over multiple generations. A more detailed evaluation of the MON 89788 event can be found in a previous EFSA opinion (EFSA 2008).

3.1.3. Transgenic constructs in MON 87701 × MON 89788

The integrity of the individual inserts present in this soybean was investigated using Southern analyses[9]. This involved the use of DNA probes specific for MON 87701 and MON 89788 inserts and the use of restriction enzymes that allowed the structures of the inserts, including the junction regions, to be determined within the stack. The predicted DNA hybridisation patterns from each single event were retained in the MON 87701 × MON 89788 stack, demonstrating that integrity of the inserts was maintained.

3.1.4. Information on the expression of the insert

The levels of newly expressed proteins Cry1Ac and CP4 EPSPS of soybean MON 87701 × MON 89788 were analysed by enzyme-linked immunosorbent assay (ELISA)[10]. Tissue samples for analysis were collected from five field trials conducted in Argentina during 2007/2008. The trials were located in major soybean-growing regions of Argentina and provided a variety of environmental conditions. Each trial included appropriate comparators (MON 89788 and MON 87701 as positive controls, and a conventional soybean variety with a genetic background similar to soybean MON 87701 × MON 89788 as a negative control). Over-season leaf (OSL 1–4), forage, root and seed tissues were collected from each replicated plot at all field sites.

The scope of the application covers food and feed uses and import and processing, therefore protein expression data related to the seeds are considered most relevant and are summarised in Table 1. Levels of proteins in the stacked line were comparable to levels in the single events. Although some statistically significant differences were found, these differences were small or not consistent across the growing season.

[9] Technical Dossier/Section D.2.
[10] Technical Dossier/Section D.3.

Table 1 Summary of protein levels in seeds of soybean MON 87701 × MON 89788 (µg/g dry weight)

		MON 87701 × MON 89788	MON 87701	MON 89788
Cry1Ac	Mean	7.9	5.1	N/A
	Range	4.5–12	3.6–6.7	
CP4 EPSPS	Mean	160	N/A	160
	Range	74–300		38–300

N/A, not assayed.

3.1.5. Inheritance and stability of inserted DNA

The genetic stability of the inserted DNA in events MON 89788 and MON 87701 has been demonstrated previously (EFSA 2008; 2011a). In soybean MON 87701 × MON 89788 the two inserts are combined. The Southern data show that the integrity of the inserts present in the single events is retained in MON 87701 × MON 89788. Furthermore, each of the traits has been conserved in this soybean.

3.2. Conclusion

As conventional breeding methods were used in the production of soybean MON 87701 × MON 89788, no additional genetic modification was involved. Southern analyses demonstrated that the integrity of the inserts in the MON 87701 and MON 89788 events was retained in soybean MON 87701 × MON 89788. The levels of Cry1Ac and CP4 EPSPS proteins in the seeds (and all other tissues examined) of soybean MON 87701 × MON 89788 have been demonstrated to be comparable with those in the single events. The EFSA GMO Panel concludes that the molecular characterisation does not indicate a safety concern.

4. COMPARATIVE ANALYSIS

4.1. Evaluation of relevant scientific data

4.1.1. Summary of the previous evaluation of the single events

4.1.1.1. MON 87701

The EFSA GMO Panel has already given an opinion on the insect-resistant soybean MON 87701 (EFSA 2011a). In the compositional studies, the GM soybean MON 87701 was compared with the Asgrow variety A5547, which is a non-GM soybean variety with background genetics similar to MON 87701. The compositional seed and forage data for these soybean materials were collected in field trials in the USA (2007) and Argentina (2007/2008), each season/year at five different geographical sites. Each field trial included soybean MON 87701, the conventional counterpart (A5547) and four different commercial non-GM soybean varieties per field trial site. In total, 20 commercial soybean varieties were used as reference lines to provide data on the natural variation in the composition of this food and feed plant. The results of the compositional analysis of materials from these field trials have been published (Berman et al. 2009). A summary of these studies is given below.

The constituents analysed were the same as in the study of soybean MON 87701 × MON 89788 (see section 4.1.3). In both years/seasons of field trials, analysis of the data across sites revealed no statistically significant differences in the level of analysed constituents in forage between soybeans MON 87701 and A5547. In the corresponding analysis of data from seeds, 15 statistically significant differences were observed in the material from the field trials in 2007 in the USA (the proximates protein and carbohydrates; the amino acids alanine, glycine, histidine, isoleucine, leucine, lysine, serine, threonine and valine; the fatty acid behenic acid; vitamin E; trypsin inhibitor; and daidzein), whereas the level of only four of the constituents differed between seeds of soybean MON 87701 and A5547 in material from the Argentinian field trials in the season 2007/2008 (the amino acid tryptophan, the fatty acid linolenic acid, vitamin E and stachyose). Apparently, the increase in the nine amino acids in the field trials in the USA reflected the increased protein content of the seed. Nevertheless, the

statistically significant differences between soybean MON 87701 and its conventional counterpart were usually small and inconsistent (observed at a few of the field trial sites), and measured levels were with one exception (a single low lectin value) within the range defined by the commercial non-GM varieties included in the studies or reported by the ILSI database (2006) or the USDA-ISO (2006) isoflavone database. Thus, the only constituent for which the difference was consistent (four of the five field trial sites in the USA and five of the five sites in Argentina) and reached some level (around 25%) was vitamin E. When statistically analysed over all field trial sites each growing season, vitamin E levels were increased both in 2007 (7.69 vs 6.24 mg/100 g dry weight) and in the season 2007/2008 (4.40 vs 3.42 mg/100 g dry weight). Although the vitamin E level was increased by on average around 25 % in soybean MON 87701, the level is still within the range of values commonly observed in conventional commercial non-GM soybean varieties, as defined by the reference lines, and by the ILSI crop composition database (ILSI 2006). The applicant provided information on the agronomic performance and phenotypic characteristics of soybean MON 87701 and soybean A5547 (conventional counterpart) from 16 field trial sites in the USA in 2007 and eight field trial sites in Argentina in the season 2007/2008. These field trials also included several commercial non-GM 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 label rates. 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 content (11.0 % vs 11.6 %) were reduced in soybean MON 87701, and test weight (171.3 vs 169.2 g/250 ml) increased, but these significant differences were found at only two, five, and three, respectively, out of the eight field trial sites in the season 2007/2008. Whereas seed moisture content and test weight were within the range of values defined by the commercial non-GM soybean varieties, the early stand count for soybean MON 87701 (96.9 plants) was slightly below the range for the reference varieties (103.8–204.0 plants). The applicant gave a plausible explanation in that the lower early stand count, which did not influence yield and final stand count, could be due to the different climatic conditions under which the seeds used for the present field trials were produced (MON 87701 seeds were produced in the USA and the A5547 seeds in Puerto Rico). Separate studies have revealed no difference in the percentage of viable pollen produced, pollen diameter and pollen morphology between soybean MON 87701 and its conventional counterpart A5547. Nor were any relevant alterations in germination characteristics observed between soybeans MON 87701 and A5547.

Compositional equivalence between soybean MON 87701 in a different genetic background than in that under consideration here and commercial non-GM soybean varieties has been confirmed in studies on forage and seeds harvested in Brazil (Berman et al. 2010).

The EFSA GMO Panel considered the total set of compositional, phenotypic and agronomical data supplied, and the statistically significant differences between soybean MON 87701 and its conventional counterpart A5547, and concluded that soybean MON 87701 is not different compositionally from its conventional counterpart, except that is has an increased vitamin E content and expresses the Cry1Ac protein. Other than the latter attribute, soybean MON 87701 is compositionally equivalent to commercial non-GM soybean varieties. Regarding agronomic performance and phenotypic characteristics, the Panel identified no difference between soybean MON 87701 and its conventional counterpart and a large set of non-GM soybean varieties that is likely to be biologically relevant.

4.1.1.2. MON 89788

The EFSA GMO Panel has already given an opinion on the glyphosate-tolerant soybean MON 89788 (EFSA 2008). In the compositional studies, the GM soybean MON 89788 was compared with the Asgrow variety A3244, which is a conventional non-GM soybean variety with background genetics similar to MON 89788. Soybean MON 89788 was treated with glyphosate-based herbicides at the recommended dose for commercial use, and the conventional counterpart A3244 and 12 commercial non-GM soybean varieties (included in the study to establish the natural variation in the level of soybean constituents) were treated with other commercial herbicides. The field trials were carried out in Argentina in the season 2004/2005 and in the USA in 2005 at five

different geographical sites each season/year. The results of the compositional analysis of materials from these field trials have been published (Lundry et al. 2008). A summary of these studies is given below.

The constituents analysed for soybean seeds and forage were the same as in the study of soybean MON 87701 × MON 89788 (see section 4.1.3). Statistical differences in the level of analysed constituents between soybeans MON 89788 and A3244 across field trial sites were observed for four constituents in one of the two seasons. These constituents were the moisture content of the forage and the levels of daidzein, glycitein and vitamin E in the seeds. Differences relative to the control were small (–1.6 %, –7.4 %, –10.6 % and 7.4 %, respectively), and were well within the natural variation calculated from the occurrence of these constituents in the 12 commercial non-GM soybean varieties. They also fell within the natural variation of these constituents in soybeans described in the USDA-ISO (2006) isoflavone database. When statistically analysed per site, the level of the first three of these four constituents was significantly altered at one of the five trial sites, whereas for the fourth, vitamin E, the level was not significantly altered at a single site. The statistical analysis of compositional data of soybean MON 89788 and A3244 within sites showed no consistent alteration in the level of the studied components between sites or between growing seasons. Furthermore, the differences were generally small and fell within the range of natural variation calculated from the occurrence of these constituents in conventional soybean varieties.

The applicant also supplied data from field trials in the USA in 2006 (eight sites) and 2007 (two sites), in which soybean MON 89788 sprayed with glyphosate-based herbicides was compared with unsprayed MON 89788 in order to confirm that the spraying regime had no unexpected influence on the soybean composition. No non-GM controls were included in these field trials. The only soybean constituent the level of which was statistically significantly different between unsprayed and sprayed soybean MON 89788 in both years of these field trials was stachyose. However, sprayed plants contained higher levels in 2006 and reduced levels in 2007.

Harrigan et al. (2010) have discussed some of the compositional data obtained from soybean MON 89788, its conventional counterpart and commercial non-GM soybean varieties in more detail. The EFSA GMO Panel concluded that soybean MON 89788 is compositionally equivalent to the conventional counterpart soybean A3244 and other conventional soybean varieties, except that it expresses the introduced trait (EFSA 2008).

The applicant provided information on the agronomic performance and phenotypic characteristics of soybean MON 89788 and soybean A3244 (control) from 17 field trials performed in the USA in 2005. These studies also gave information on reproduction, dissemination and survival of these soybeans, as well as on three or four commercial non-GM soybean varieties for each trial site (in total 23 varieties for all trial sites). The only difference between soybeans MON 89788 and A3244, confirmed by statistical analysis over all trial sites, was plant height, which was lower in soybean MON 89788 than in the control (77.9 vs 82.0 cm). This reduction in plant height was noted at four of the seven sites but was always within the natural variation of the commercial soybean varieties (48.8 to 108.2 cm). As the magnitude of the difference was small (around 5.3 %), the plant height fell within the normal variation and no ecological risks could be linked to the reduction in height, the Panel found this difference to be of no biological importance. No difference in pollen morphology and viability was observed.

Compositional equivalence between soybean MON 89788 in genetic backgrounds other than in the original application and commercial non-GM soybean varieties has been confirmed in studies on forage and seeds harvested in Brazil (Berman et al. 2010). In addition, De Vries and Fehr (2011) studied a selection of compositional and agronomic parameters in 27 back-crossed MON 89788 glyphosate-tolerant lines and 27 back-crossed glyphosate-sensitive lines obtained from populations segregating from soybean MON 89788 in three genetic backgrounds and grown at four field trial sites in the USA. Although statistically significant differences in the mean values for some of the studied parameters were observed between soybean MON 89788 and its non-GM comparator in some of the genetic backgrounds, these differences were small, not consistent over the various genotypes and considered not to be biologically relevant. The investigators concluded that it would be possible to select glyphosate-tolerant (MON 89788) and glyphosate-sensitive lines with comparable performance from soybean populations developed by crossing a glyphosate-sensitive parent with a glyphosate-tolerant parent carrying the MON 89788 event (De Vries and Fehr 2011).

The EFSA GMO Panel considered soybean MON 89788 and its non-GM counterpart to be compositionally and agronomically equivalent to conventional soybean lines, except that soybean MON 89788 expresses the CP4 EPSPS protein rendering the plant glyphosate tolerant. The comparative analysis of soybean MON 89788 with the non-GM variety A3244 and other conventional soybean varieties provided no indication of unintended effects resulting from the genetic modification. Data published on soybean MON 89788 after the EFSA GMO Panel gave its opinion on this GM crop confirm the interpretation of the Panel.

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

The application EFSA-GMO-NL-2009-73 for food and feed use, import and processing of soybean MON 87701 × MON 89788 within the EU presented compositional data from seed and forage material collected in field trials in the USA (2007) and Argentina (2007/2008). These field trials compared the composition of soybean MON 87701 × MON 89788 with a comparator having a comparable genetic background. The comparator was the Asgrow variety A5547, which was the commercial soybean variety originally used in the transformation to establish transformation event MON 87701.

In both years/seasons, the field trials were performed at five separate sites, all of which were representative of the soybean cultivation areas of the countries. The plots designed to supply material for comparative compositional studies included soybean MON 87701 × MON 89788 treated with glyphosate-based herbicides (and, in addition, maintenance pesticides) and the comparator (A5547) and four different commercial non-GM soybean varieties treated with required maintenance pesticides (referred to as untreated). On average, glyphosate was applied at a later stage of soybean growth in the South American field trials than in the North American field trials. The plots designed for agronomic, phenotypic and ecological studies were not sprayed with glyphosate. Altogether 20 commercial non-GM soybean varieties[12] were the reference lines used to provide data on the natural variation in composition of this food and feed plant. The reference lines were checked for natural contamination with the MON 87701 and MON 89788 events. One of the replicates of A5547 at one of the field trial sites in the USA and Argentina contained unacceptably high levels of unintended traits and was excluded from the study. At each field trial site, soybean MON 87701 × MON 89788, the comparator and the commercial non-GM lines were planted following a randomised complete block design with three replicates at each site. Whereas all replicates of soybean MON 87701 × MON 89788 and its comparator were chemically analysed for selected soybean constituents, only one of the replicates of the commercial non-GM varieties was analysed.

As there were no compositional data on soybean MON 87701 × MON 89788 treated with maintenance pesticides other than glyphosate to be compared with soybean A5547 treated with maintenance pesticides, and no agronomic and phenotypic analysis of soybean MON 87701 × MON 89788 treated with glyphosate and maintenance pesticides to be compared with soybean A5547 treated with maintenance pesticides, the EFSA GMO Panel requested additional data on the composition, agronomic and phenotypic characteristics and ecological interaction of soybean MON 87701 × MON 89788 compared with its comparator A5547. The applicant supplied compositional data on soybean MON 87701 × MON 89788 and its comparator not treated with glyphosate from the field trial performed in Argentina in 2007/2008 (similar data were not available from the field trials in the USA in 2007) and agronomic and phenotypic data for soybean MON 87701 × MON 89788 and its comparator treated and not treated with glyphosate from field trials in the USA in 2009[13]. The latter studies also used eight commercially available soybean varieties[14] as reference materials.

[11] Technical dossier/Section D7.2

[12] The commercial non-GM varieties in the field trials in the USA were A5843, A5959, CMA5804AOC, H6686, UA 4805, Ozark, Anand, Hornbeck C5894, A5560, CMC5901COC, A5403, LEE 74, A4922, H4994, H5218, A5427, DP 5989, Hutcheson, Fowler, and USG 5601T. The same commercial non-GM varieties were used in the field trials in Argentina, except for H6686, for which USG 5002T was substituted.

[13] Additional information, November 2011.

[14] The commercial non-GM varieties used in the field trials in the USA in 2009 were Ozark, Anand, H5218, A5427, Teejay, Jake Fowler and USG 5601T.

4.1.3. Compositional analysis[15]

Soybean seeds were harvested and analysed for proximates (protein, fat, ash and moisture), carbohydrates by calculation, fibre fractions (acid detergent fibre (ADF) and neutral detergent fibre (NDF)), amino acids, fatty acids, the micronutrient 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, including those recommended by OECD (2001), were analysed in the material harvested in field trials in the USA, 57 in seeds and seven in forage. The data on each analyte were statistically analysed for potential difference in levels between soybean MON 87701 × MON 89788 and its comparator 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 minor constituents and frequently occurred at levels below the limit of quantification; when this occurred in more than 50 % of the samples, the analyte was omitted from the statistical analysis. In cases in which constituent levels were statistically significantly different between seeds of soybean MON 87701 × MON 89788 and its comparator, the level was compared with the levels occurring in the commercial non-GM soybean varieties included in the study, as well as with ranges of soybean constituent levels published in the scientific literature and in the ILSI crop composition database (ILSI 2006).

When the compositional data for seed samples from the field trials in the USA (soybean MON 87701 × MON 89788 sprayed with glyphosate, the comparator untreated) were evaluated across sites, a statistically significant difference between soybean MON 87701 × MON 89788 and its comparator was found for 20 analytes: the proximate protein; the amino acids alanine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, proline, serine, threonine and valine; the fatty acids palmitic acid, stearic acid, linolenic acid and arachidic acid; and lectin, daidzein and genistein. For forage only the level of total protein differed significantly between soybean MON 87701 × MON 89788 and its comparator. Apparently, the increase in the 12 amino acids reflected the increased protein content of the seed. The evaluation per site revealed that not all of the differences observed for the across-site analysis occurred at all of the individual sites. The statistically significant differences between soybean MON 87701 × MON 89788 and its comparator were usually small, and in all cases, except one, the levels detected were within the range found in the commercial non-GM varieties included in the study. The exception was a single lectin value in one of the three replications of soybean MON 87701 × MON 89788 at a single field trial site. The EFSA GMO Panel concluded that this was an incidental deviation not reflecting the characteristics of soybean MON 87701 × MON 89788. Also, the 26 additional statistically significant differences identified in the per-location statistical analysis of other soybean constituents were small and did not raise concern.

The statistical evaluation of compositional data of seed samples from field trials in Argentina across sites, revealed statistically significant differences between glyphosate-sprayed soybean MON 87701 × MON 89788 and its untreated comparator for 11 analytes: the proximate ash; the two amino acids glutamic acid and leucine; the fatty acids stearic acid, linoleic acid and arachidic acid; and vitamin E, stachyose, daidzein and genistein. The evaluation per site illustrated that, among the 11 constituents that were significantly different in soybean MON 87701 × MON 89788 and the comparator in the across-site analysis, four showed a statistically significant difference at one site, three at two sites, two at three sites and one at four of the five sites. Only the level of acid detergent fibre differed significantly between soybean MON 87701 × MON 89788 and its comparator in forage. The statistically significant differences between soybean MON 87701 × MON 89788 and its comparator were usually small and inconsistent, and in all cases the levels registered were within the range described by the commercial non-GM varieties included in the study and the range reported by the ILSI database (2006) or the USDA-ISO (2006) isoflavone database. In addition, the 16 additional statistically significant differences identified in the per-location statistical analysis of other soybean constituents were small, and all but two were within the range defined by the 20 soybean non-GM reference varieties. Both individual values lying outside the range defined by the non-GM varieties were slightly higher moisture contents of seeds of the comparator A5547: 10.22 % and 10.97 % fresh weight, compared with the range in moisture content of the reference lines (6.88–10.06 % fresh weight). Thus, the statistical analysis comparing compositional data from forage and seeds of

[15] Technical dossier/Section D7.1

glyphosate-treated soybean MON 87701 × MON 89777 and the untreated comparator harvested in field trials in the USA and Argentina, respectively, identified 20 differences between the genetically modified soybean and its comparator in 2007 and 11 in 2007/2008. Six constituents were altered in both growing seasons. These were glutamic acid (altered in one of the ten field sites), leucine (two sites), stearic acid (eight sites), arachidic acid (five sites), daidzein (five sites) and genistein (four sites). However, the levels of these seed constituents were not consistently different across individual field trial sites (figures given within brackets above) and were within the range of values commonly observed in conventional soybean varieties, as defined by the commercial non-GM varieties. There were no consistent statistically significant differences in forage parameters between soybean MON 87701 × MON 89777 and the comparator. Furthermore, the statistically significant differences between these materials observed in only one of the seasons of field trials were small and levels were within the range observed in the commercial non-GM varieties. As the protein content of soybean MON 87701 × MON 89788 was higher than in its comparator (being statistically significant in one of the growing seasons), the difference in glutamic acid and leucine could be foreseen. The level of individual fatty acids varies considerably depending on environmental conditions, as is demonstrated by the wide range in content of the various fatty acids in the commercial non-GM material. This is also the case for the flavonoids daidzein and genistein (USDA-ISO 2006). The approximately 25 % increased level of vitamin E observed in one of the parental soybean events, MON 87701 in the A5547 background (EFSA 2011a), was not observed in soybean MON 87701 × MON 89788.

On request from the EFSA GMO Panel, the applicant supplied compositional data from forage and seed samples from the field trials in Argentina 2007/2008 in which both soybean MON 87701 × MON 89788 and its comparator were not treated with glyphosate (sprayed with maintenance pesticides only)[16]. Analysis of the seed data across sites revealed statistically significant differences between the two soybean materials for 12 analytes: the proximates moisture and ash; the amino acid tryptophan; the fatty acids stearic acid, oleic acid, linoleic acid and arachidic acid; and vitamin E, raffinose, stachyose, daidzein and genistein. The evaluation per site illustrated that among these 12 constituents, four showed statistically significant differences at one site, three at two sites, two at three sites and three at four of the five sites. Again in this case the statistically significant differences between soybean MON 87701 × MON 89788 and its comparator were small (never above 20 %) and in all cases within the range found in the commercial non-GM varieties included in the study and the range reported by the ILSI database (2006) or the USDA-ISO (2006) isoflavone database. The level of none of the constituents analysed in forage differed between soybean MON 87701 × MON 89788 and its comparator.

In conclusion, the only statistically significant differences across locations between soybean MON 87701 × MON 89788 and its comparator that were consistently observed in both the USA and Argentina across the seasons were changes in the level of some fatty acids and increased levels of daidzein and genistein. These differences were small and not considered biologically relevant. Moreover, the values reported fell within the range defined by the natural variation of these constituents in commercial non-GM soybean varieties grown in the same field trials.

The EFSA GMO Panel considered the total set of compositional data supplied and the observed statistically significant differences between soybean MON 87701 × MON 89788 and its comparator, in the light of the field trial design, measured biological variation and the level of the studied compounds in commercial non-GM soybean varieties, and concluded that no biologically relevant differences were identified in the compositional characteristics of soybean MON 87701 × MON 89788 in comparison with its comparator soybean A5547 and that its composition fell within the range of non-GM soybean varieties, except that is expresses the CP4 EPSPS and Cry1Ac proteins.

4.1.4. Agronomic traits and GM phenotype[17]

The applicant performed a comparative assessment of the phenotypic and agronomic characteristics of soybean MON 87701 × MON 89788 and its comparator (A5547) based on field trials at eight sites in Argentina in the season 2007/2008. At each site a randomised complete block design was used, with four replications at three of the sites and three replications at the other five sites. These field trials also included several commercial non-GM

[16] Additional information November 2011.
[17] Technical dossier/Section D4.

soybean varieties[18] (four per site) used as reference material to estimate a range of baseline values among commercial non-GM soybean varieties for each studied phenotypic and agronomic parameter. All materials were grown under normal agronomic conditions for the geographical region; glyphosate-based herbicides were not used. 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 content, 100-seed weight, test weight (g/250 ml) and yield. In the phenotypic comparison around half of the parameters studied differed between soybean MON 87701 × MON 89788 and A5547. Early stand count (140.6 vs 105.9 plants in defined rows), final stand count (130.0 vs 97.1 plants in defined row), test weight (171.8 vs 169.2 g/250 ml) and yield (2.8 vs 2.5 t/ha) were increased, whereas lodging (2.4 vs 3.3 scale points), grain moisture content (10.9 % vs 11.6 % fresh weight) and 100-seed weight (15.3 vs 16.0 g) were decreased. Of these differences early and final stand count were significantly higher at seven out of the eight field trial sites studied. Test weight was increased at six of eight sites and yield at three of eight sites. A reduction in seed moisture content was observed at seven of eight sites, a lower 100-seed weight at four of eight sites, and reduced lodging at five of eight sites. As the measured endpoint for all parameters showing a statistically significant difference between soybean MON 87701 × MON 89788 and its comparator was within the range in levels of these constituents in soybean reference varieties in the combined site analysis, the applicant argued that no biologically relevant difference in terms of increased potential for the soybean to become a weed between soybean MON 87701 × MON 89788 and its comparator was identified, except the expected difference in tolerance to glyphosate. No developmental differences in categorical parameters (flower colour, plant pubescence and plant growth stage) were observed between soybean MON 87701 × MON 89788 and its comparator.

In the field trials conducted in Argentina in the season 2007/2008, plant response to abiotic stressors and the effects of disease damage were measured four times during the growing season at all eight field trial sites, whereas arthropod damage and abundance were evaluated 15 and nine times, respectively, during the growing season at three of the eight sites. The stressors were defined by experts at each field trial site and varied between sites. A difference between soybean MON 87701 × MON 89788 and its comparator was noted in one of the 192 comparisons made for abiotic stress and plant disease damage. This was caused by soybean mosaic virus at one of the field trial sites during observation 2 (MON 87701 × MON 89788 none vs A5547 slight). This disease damage category was within the range of damage observed among the reference soybean varieties. Regarding arthropod damage, no statistical significant difference was detected between soybean MON 87701 × MON 89788 and the comparator for 23 out of 32 comparisons. In eight of the nine cases in which a difference was detected, it was the result of reduced damage in soybean MON 87701 × MON 89788 caused by lepidopteran pests, which was expected as this GM soybean carries an insect protection trait targeted against lepidopteran pests. The other difference detected was reduced damage by stink bugs in soybean MON 87701 × MON 89788 than in A5547; however, notably in this case the stink bug damage was within range in the reference soybean varieties and observed on only one occasion. There was no difference in arthropod abundance between soybean MON 87701 × MON 89788 and its comparator regarding several investigated species.

On request from the EFSA GMO Panel for additional agronomic and phenotypic data, the applicant supplied data from field trials in the USA in 2009 in which soybean MON 87701 × MON 89788 sprayed with glyphosate and maintenance pesticides[19], or sprayed with maintenance pesticides only[20], was compared with the comparator A5547 sprayed with maintenance pesticides. Of the five field trials initiated in the soybean-growing regions of the USA, one site (Arkansas) was dropped from the study owing to poor germination and emergence as a result of excessive rain. Differences were observed in two agronomic characteristics between the glyphosate-sprayed soybean MON 87701 × MON 89788 and the comparator, namely seedling vigour and final stand count. The differences, however, were not large, and measured values fell within the range found in commercial non-GM soybean varieties grown in the same field trials.

[18] A total of 20 commercial reference varieties were evaluated (USG 5002T, Asgrow A5427, Hornbeck C5894, Asgrow A5959, Hartz H5218, Asgrow A5403, CMA 5804A0C, DP 5989, Ozark (5.2), Annand (5.4), Asgrow A5843, CMC 5901C0C, UA 4805, A5560, LEE 74, A4922, H4994, Hutcheson, USG 5601T, Fowler).
[19] Additional information November 2011.
[20] Additional information November 2011.

When plants were not treated with glyphosate, one statistically significant difference was observed between soybean MON 87701 × MON 89788 and its comparator – and that was a higher stand count. The increased mean values in stand count were within the range observed in commercial non-GM soybean varieties grown in the same field trials.

It is concluded that crossing insect-resistant soybean MON 87701 with glyphosate-tolerant soybean MON 89788 to produce the stacked soybean MON 87701 × MON did not result in any consistent changes in phenotypic and agronomic characteristics, as compared with its comparator, with the exception of a small increase in final stand count which is not considered biologically relevant by the EFSA GMO Panel.

4.2. Conclusion

The EFSA GMO Panel concludes that no biologically relevant differences were identified in the composition or agronomic and phenotypic characteristics of soybean MON 87701 × MON 89788, as compared with the comparator soybean A5547, and that the composition of soybean MON 87701 × MON 89788 fell within the range observed in non-GM soybean varieties, except that it expresses the CP4 EPSPS and Cry1Ac proteins. Based on the assessment of the data available, the EFSA GMO Panel is of the opinion that crossing soybean MON 87701 and soybean MON 89788 to produce soybean MON 87701 × MON 89788 does not result in interactions that cause compositional, agronomic or phenotypic changes that would raise safety concerns.

5. FOOD/FEED SAFETY ASSESSMENT

5.1. Evaluation of relevant scientific data

5.1.1. Summary of the previous evaluation of the single events

5.1.1.1. MON 87701

The EFSA GMO Panel has already given an opinion on the safety assessment of soybean MON 87701 (EFSA 2011a). In short, soybean MON 87701 expresses a Cry1Ac protein from an introduced gene (*cry1Ac*) derived from *Bacillus thuringiensis* subsp. *kurstaki*. An *Escherichia coli*-produced Cry1Ac protein was used for safety studies after it had been demonstrated experimentally that it was structurally similar and physicochemically/functionally equivalent to the Cry1Ac protein extracted from soybean event MON 87701. No toxicity of the Cry1Ac protein was observed up to the highest doses tested (1460 mg/kg body weight in males and 1290 mg/kg body weight in females) in an acute oral toxicity study in mice. The Cry1Ac protein was shown to be quickly degraded in simulated gastric fluid, and all transiently appearing fragments were quickly degraded on subsequent exposure to simulated intestinal fluid. Bioinformatics studies demonstrated that the Cry1Ac protein showed homology only with other Cry proteins not toxic to mammals. The newly expressed Cry1Ac protein was shown to be unlikely to be an allergenic protein and the whole GM soybean MON 87701 unlikely to differ in allergenic potential from that of commercial non-GM soybeans. Two 90-day feeding studies in rats with diets including 15 % or 30 % toasted and defatted soybean meal prepared from soybean MON 87701 or the conventional counterpart (A5547) indicated no toxicity. In addition, a 42-day broiler chicken 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 commercial non-GM soybean varieties.

5.1.1.2. MON 89788

The EFSA GMO Panel has already given an opinion on the safety assessment of soybean MON 89788 (EFSA 2008). In short, soybean MON 89788 expresses the CP4 EPSPS protein. An *E. coli*-produced CP4 EPSPS protein was used for safety studies after it had been demonstrated experimentally that the microbially produced protein is structurally similar and physicochemically/functionally equivalent to the CP4 EPSPS protein extracted from soybean event MON 89788. No toxicity of the CP4 EPSPS protein was observed up to the highest dose tested (572 mg/kg body weight) in an acute oral toxicity study in mice. The CP4 EPSPS was shown to be quickly degraded in simulated gastric fluid, and a little less quickly degraded in simulated intestinal fluid. A quick

degradation of the CP4 EPSPS protein in simulated gastrointestinal fluids has been confirmed (Shim et al. 2010). Bioinformatics studies demonstrated that the CP4 EPSPS protein show no homology to known toxic or allergenic proteins. A 90-day feeding study in rats with diets including 5 % or 15 % processed meal of soybean MON 89788 or the conventional counterpart (A3244) indicated no toxicity. In addition, a recently published study showed that feeding a diet containing about 38 % soybean flour from MON 89788 (for 182 days) to male Wistar rats had comparable responses to feeding a diet containing the same amount of flour from soybean A3244 (Tutelyan et al. 2010; Tyshko et al. 2010). The original application contained a 42-day feeding study on broiler chickens, which has now been published and shows that soybean MON 89788 is as nutritionally wholesome as the conventional counterpart and commercial non-GM soybean varieties (Taylor et al. 2007). Using extracts from soybean MON 89788, the conventional counterpart (A3244), and commercial non-GM soybean varieties, and sera from non-allergic and soybean-allergic patients, it was demonstrated that it is unlikely that the overall allergenicity of the whole GM soybean MON 89788 is different from that of conventional soybeans.

5.1.2. Product description and intended use[21]

The scope of application EFSA-GMO-NL-2009-73 is for food and feed use, import and processing of soybean MON 87701 × MON 89788 within the EU. Thus, soybean MON 87701 × MON 89788 will be imported into the EU mixed with other soybean varieties and used as food or feed or for the production of a large number of derived products, in the same way 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 into soybean products for human consumption, including flours, soybean protein concentrates and various textured products simulating meats, seafoods and cheeses. The rest of the toasted defatted soybean meal goes into animal feed, mainly for poultry, pigs and cattle in the EU (OECD 2001). Whole soybeans are used to produce soy sprouts and baked and roasted soybeans. There is also a limited direct use for soybeans as animal feeds. The genetic modification events present in soybean MON 87701 × MON 89788 result in the expression of two new proteins, one being the Cry1Ac protein, which confers protection against lepidopteran pests such as velvet bean caterpillar (*Anticarcia gemmatalis*), soybean looper (*Pseudoplusia includens*), bean shoot borer (*Epinotia aporema*) and sunflower looper (*Rachiplusia nu*), and the other being the CP4 EPSPS enzyme, which is less sensitive to glyphosate (which inhibits the synthesis of aromatic amino acids) than the endogenous plant EPSPS enzyme and, therefore, allows soybean MON 87701 × MON 89788 to produce aromatic amino acids and grow normally in the presence of glyphosate herbicides. Thus, the genetic modifications are intended only to improve agronomic performance and are not intended to influence the nutritional aspects, the processing characteristics and the overall use of soybean as a crop.

5.1.3. Effects of processing[22]

Soybean MON 87701 × MON 89788 will be used for production and manufacturing of food and feed products in the same ways as any other commercial soybean variety. Taking into account the compositional analysis, providing no indication of relevant compositional changes except for the stacked soybean expressing the CP4 EPSPS and Cry1Ac proteins, the Panel has no reason to assume that the characteristics of soybean MON 87701 × MON 89788, and derived processed products, would be any different from those of the corresponding products derived from soybean MON 87701, soybean MON 89788 and conventional soybean varieties. The processing of soybean MON 87701 × MON 89788 will be no different from the processing of conventional soybeans. Thus, solvent extraction, hard pressing and extrusion result in various types of soybean oil products used as food, soybean meal for animal feed, protein products usually used as feed but to some extent also as food, and various specialised products such as lecithin. Heat treatment of soybean MON 89788 (190 ºC for 30 min), simulating the process used in commercial soybean processing, reduced the amount of immune-detectable CP4 EPSPS protein present in soybean MON 89788 to levels below the limit of detection, thus representing a more than 97 % reduction in the quantity of detectable CP4 EPSPS compared with the unheated MON 89788 sample. A similar study on heat-treated soybean MON 87701 (190 ºC for 15.5 min) reduced also the quantity of immune-detectable Cry1Ac protein to levels below the limit of detection, corresponding to a reduction of at least 94 % compared with the unheated MON 87701. It was suggested that the losses are likely to

[21] Technical dossier/Section D7.7.
[22] Technical dossier/Section D7.6.

be due to protein degradation and/or aggregation into an insoluble complex as a result of the heat treatment. The EFSA GMO Panel finds it likely that similar heat treatments of soybean MON 87701 × MON 89788 will result in corresponding reductions in the amount of immune-detectable CP4 EPSPS and Cry1Ac protein in the heat-treated product.

5.1.4. Toxicology[23]

5.1.4.1. Toxicological assessment of expressed novel proteins in soybean MON 87701 × MON 89788

No new genes, in addition to those occurring in the parental soybean varieties, have been introduced in soybean MON 87701 × MON 89788. The CP4 EPSPS protein expressed in soybean MON 89788 has been evaluated for its safety previously (EFSA 2008), and no safety concerns were identified. This was confirmed in an updated bioinformatics study in which the amino acid sequence of the CP4 EPSPS protein was compared with amino acid sequences available in databases containing toxic proteins. The EFSA GMO Panel is not aware of any other new information that would change this conclusion. The Cry1Ac protein expressed in soybean MON 87701 was more recently evaluated for its safety (EFSA 2011a), and in this case too no safety concerns for humans or animals were identified. Quantification of expression levels of the Cry1Ac and CP4 EPSPS proteins in various tissues of soybean MON 87701 × MON 89788, MON 87701 and MON 89788 revealed comparable expression levels in the stacked hybrid as compared with the expression levels in the parental events (at most a twofold difference).

The EFSA GMO Panel has reviewed all the data available for soybean MON 87701 × MON 89788, both for the single events and for the newly expressed proteins Cry1Ac and CP4 EPSPS, including information provided by the applicant in response to questions from the Panel, and considers that interactions between the single events that might impact on food and feed safety are unlikely.

5.1.4.2. Toxicological assessment of new constituents other than proteins

No new constituent, other than the Cry1Ac and CP4 EPSPS proteins, is expressed in soybean MON 87701 × MON 89788, and no relevant changes in the composition of soybean MON 87701 × MON 89788 were detected by the compositional analysis.

5.1.4.3. Toxicological assessment of the whole GM food/feed

Both soybeans MON 89788 and MON 87701 have previously been found as safe for human and animal consumption as their corresponding conventional counterparts and commercial non-GM soybean varieties (EFSA 2008, 2011a).

A molecular characterisation undertaken on soybean MON 87701 × MON 89788 identified no altered stability of the single soybean events (see section 3.1.5) when these were brought together by crossing, and expression analysis of the Cry1Ac and CP4 EPSPS proteins revealed no relevant change in expression levels in soybean MON 87701 × MON 89788 compared with the single soybean events MON 87701 and MON 89788, respectively (see section 3.2). As no biologically relevant differences were identified in the compositional characteristics of soybean MON 87701 × MON 89788 in comparison with non-GM soybean varieties, except that it expresses the CP4 EPSPS and Cry1Ac proteins, and an assessment found no indication for interaction between the single events that could influence the safety of soybean MON 87701 × MON 89788 for humans and animals, the EFSA GMO Panel is of the opinion that no additional animal safety studies are required.

5.1.5. Allergenicity[24]

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

[23] Technical dossier/Section D7.8.
[24] Technical dossier/Section D7.9

reactions in already sensitised persons, and whether the transformation may have altered the allergenic properties of the modified food.

5.1.5.1. Assessment of allergenicity of the newly expressed proteins

A weight-of-evidence approach is recommended when assessing the potential allergenicity of a newly expressed protein, taking into account all of the information obtained with various test methods, as no single experimental method yields decisive evidence for allergenicity (Codex Alimentarius 2009; EFSA 2010).

The CP4 EPSPS protein present in soybean MON 89788 and the Cry1Ac protein present in soybean MON 87701 have been evaluated previously within the assessment of the single events, and it was found unlikely that they are allergenic (EFSA 2008, 2011a). Nonetheless, the applicant supplied a bioinformatics-supported comparison of the amino acid sequence of the CP4 EPSPS protein with amino acid sequences of known allergens collected in an updated proprietary database. This study confirmed the hypothesis that CP4 EPSPS is unlikely to be an allergen. The EFSA GMO Panel has, thus, concluded that it is unlikely that these newly expressed proteins are allergenic.

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

Soybeans are common allergenic foods. Therefore, new genetically modified soybeans are assessed in order to assure that the allergenicity of the whole GM plant has not been increased by the genetic modification. Such assessments have already been performed for soybeans MON 89788 and MON 87701 (EFSA 2008, 2011a), and it was concluded that the overall allergenicity of the whole soybeans MON 89788 and MON 87701 is unlikely to be different from that of their corresponding conventional counterparts and commercial soybean varieties. On request from the EFSA GMO Panel, the applicant supplied additional data to demonstrate that the overall allergenicity of soybean MON 89788 × MON 89788 was not altered when compared with the overall allergenicity of its comparator A5547[25]. The applicant separated proteins from extracts of soybeans MON 87701 × MON 89788 and A5547 by one- or two-dimensional gel electrophoresis and identified bands of major allergenic proteins and spots of less abundant allergens by tandem mass spectrometry. On visual inspection of the intensities of the bands of the abundant α′, α, and β subunits of glycinin beta-conglycinin, the acidic and basic chains of glycinin and trypsin inhibitor on the one-dimensional gel and the less abundant spots Gly m Bd30k (P34), Gly m Bd28k and Gly m 4(SAM22) on the two-dimensional gel in soybeans MON 87701 × MON 89788 and A5547 no differences were observed. Thus, the requested study confirmed that bringing together the single soybean events MON 87701 and MON 89788 by conventional crossing to form the stacked soybean MON 87701 × MON 89788 does not result in any observable differences in allergen content between soybeans MON 87701 × MON 89788 and its comparator.

The EFSA GMO Panel considers it unlikely that potential interactions will occur in soybean MON 87701 × MON 89788 that might change the allergenicity of the whole crop.

5.1.6. Nutritional assessment of GM food/feed[26]

The applicant provided a 42-day broiler chicken feeding study (Cobb × Cobb 500) performed according to generally accepted guidelines (ILSI 2003), and consisting of nine treatment groups, all with different types of soybean meal. One group received meal of soybean MON 87701 × MON 89788, one soybean MON 87701 (not considered in this context), another soybean A5547 (a non-GM soybean with comparable background genetics to MON 87701 × MON 89788), and the other six groups meals of different commercial non-GM soybean varieties[27].

Each treatment group consisted of 60 male and 60 female broilers (in pens of 12 chickens/pen being reduced at day 7 to 10 birds/pen), which were fed starter diets (days 0–21) containing about 33 % (32.3–33.5 %) soybean meal and grower/finisher diets (days 21–42) containing 30–31 % soybean meal. The various soybean meals were

[25] Additional information November 2011.
[26] Technical dossier/Section D7.10.
[27] The non-GM soybean varieties used were Anand, Ozark, NK S38-T8, H437, NC+2A86, and NK25-J5.

characterised regarding 89 constituents before adjusted diets were formulated based on the energy and nutrient requirements for broilers according to NRC (1994). Diets were controlled for their quality with respect to pesticide and mycotoxin content, which in all cases were below threshold levels of concern for feeding studies.

The animals were weighed at the beginning of the study and after 42 days of feeding. On days 43 (males) and 44 (females), animals were sacrificed and processed for body analyses. Statistical analyses of the experimental results were performed by two-factorial analysis of variance (factors were diet and sex).The mortality rates in the different treatment groups during the first 7 days were between 0.8 % and 4.2 % (average 2.3 %; soybean MON 87701 × MON 89788 2.5 %), and were mainly due to bacterial infection and dehydration. During the remainder of the study, days 7–42, mortality rates (due to ascites and sudden death syndrome) were on average 0.6 %, ranging from 0 % to 2.0 % across the treatment groups (soybean MON 87701 × MON 89788 0 %). Surviving birds were in good health during the study. No significant effect on performance was noted. Thus, weight at the start (around 38 g) and end of the study (average 2.511–2.547 kg; soybean MON 87701 × MON 89788 2.545 kg), feed intake, weight gain and adjusted feed conversion rate (average 1.528–1.560; soybean MON 87701 × MON 89788 1.528) were comparable in broiler chickens fed diets with soybean meal from MON 87701 × MON 89788 and A5547. No relevant effects were observed on the various parameters of carcass yield or of fat, protein and moisture content of breast and thigh meat.

In conclusion, the broiler feeding study identified no relevant difference in broiler performance, carcass yield or meat composition between chickens fed diets containing soybean meal produced from soybean MON 87701 × MON 89788, the comparator A5547, or the six commercial non-GM soybean varieties. Thus, these data demonstrate that diets formulated with soybean MON 87701 × MON 89788 are as nutritious as those formulated with commercial non-GM soybean varieties.

5.1.7. Post-market monitoring of GM food/feed

The risk assessment concluded that no data have emerged to indicate that soybean MON 87701 × MON 89788 is any less safe than its comparator A5547. In addition, soybean MON 87701 × MON89788 is as nutritious as conventional soybeans. Therefore, and in line with the guidance document (EFSA 2006), the EFSA GMO Panel is of the opinion that post-market monitoring of the GM food/feed is not necessary.

5.2. Conclusion

The CP4 EPSPS protein expressed in soybean MON 89788 and the Cry1Ac protein expressed in soybean MON 87701 have been evaluated previously, and no safety concerns were identified. Given all the information provided, the EFSA GMO Panel considers that interactions between the single events that might impact on food and feed safety are unlikely. The Panel also noted that the nutritional properties of soybean MON 87701 × MON 89788 are not different from those of commercial soybean varieties. The EFSA GMO Panel considers that soybean MON 87701 × MON 89788 is as safe and as nutritious as its comparator A5547 and that it is unlikely that the overall allergenicity of the whole plant is changed.

6. ENVIRONMENTAL RISK ASSESSMENT AND MONITORING PLAN

6.1. Evaluation of relevant scientific data

The scope of this application EFSA-GMO-NL-2009-73 is for food and feed uses, import and processing, and does not include cultivation. Considering the intended uses of soybean MON 87701 × MON 89788, the environmental risk assessment is concerned with the exposure through the manure and faeces from animals fed soybean MON 87701 × MON 89788 and with the accidental release into the environment of viable grains of soybean MON 87701 × MON 89788 during transport and processing. Soybean MON 87701 × MON 89788 has been developed for tolerance to glyphosate-based herbicides and protection against certain lepidopteran pests (i.e. *A. gemmatalis*, *P. includens*, *E. aporem* and *R. nu*, which are not present in European fauna). Herbicide tolerance is conferred by the expression of the CP4 EPSPS protein. Insect resistance is achieved by the expression of the *B. thuringiensis*-derived Cry1Ac protein. 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. Evaluation of the single events

In a previous scientific opinion, the EFSA GMO Panel was of the opinion that the single soybean events MON 87701 and MON 89788 are as safe as conventional soybean with respect to potential effects on human and animal health or the environment in the context of their intended uses (EFSA 2008, 2011a). Furthermore, post-market environmental monitoring plans, including general surveillance, were proposed by the applicant and accepted by the EFSA GMO Panel for soybeans MON 87701 and MON 89788.

6.1.2. Environmental risk assessment

6.1.2.1. Unintended effects on plant fitness due to the genetic modification[28]

Cultivated soybean (*Glycine max* (L.) Merr.) is a species in the subgenus *Soja* of the genus *Glycine*. The species originated from eastern Asia and is a highly domesticated crop (Lu 2005). The major worldwide soybean producers are Argentina, Brazil, China, North Korea, South Korea and the USA. In the EU, soybean is mainly cultivated in Austria, France, Italy, Hungary and Romania (Dorokhov et al. 2004).[29] 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). 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. Similarly, insect resistance against certain lepidopteran target pests provides a potential agronomic advantage where plants are cultivated under an infestation of the target pests. However, survival of soybean plants outside cultivation where glyphosate 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 87701 × MON 89788, herbicide tolerance and insect resistance are not likely to provide a selective advantage outside cultivation or other areas 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 87701 × MON 89788 will differ from conventional soybean varieties in its ability to survive until subsequent seasons or to establish feral populations under European environmental conditions.

Field trials with soybean MON 87701 × MON 89788 were carried out by the applicant across eight locations in Argentina in 2007/2008 and across five locations in the USA in 2009 as described in section 4.1.4. As mentioned above, the combined site analysis of the 2007/2008 field data identified seven statistically significant differences in early and final stand count, test weight, yield, lodging, grain moisture content and 100-seed weight. The combined site analysis of the 2009 field data identified two statistically significant differences in seedling vigour and final stand count. 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 infestation by the specific lepidopteran pests or 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 87701 × MON 89788 in Europe will not be different from that of conventional soybean varieties.

[28] Technical dossier/sections D4, D9.1 and D9.2 and additional information, November 2011,
[29] http://epp.eurostat.ec.europa.eu/portal/page/portal/agriculture/data/database

6.1.2.2. Potential for gene transfer[30]

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

(a) Plant-to-bacteria gene transfer

Genomic 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 × MON 89788 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. This requires the presence of stretches of DNA sequences that are similar in the recombining DNA molecules and, in addition to substitutive gene replacement, facilitates the insertion of non-homologous DNA sequences if their flanking regions share sequence similarity with bacterial sequences in the recipient.

The hybrid soybean MON 87701 × MON 89788 contains genetic elements with identity or high similarity to those of bacteria. These are from MON 87701 the coding sequence of Cry1Ac, a synthetic gene that is highly similar to corresponding genes from Cry1Ac producing *B. thuringiensis*, and from MON 89788 a codon-optimised synthetic sequence of CP4 *epsps* from *Agrobacterium tumefaciens*. The flanking regions of the *cry1Ac* and CP4 *epsps* gene inserts both contain on their right border an approximately 40-bp-long sequence and on their left border for CP4 *epsps* a 150-bp-long and for *cry1Ac* a 264-bp-long sequence of the Ti-plasmid of *A. tumefaciens.*

Neither *A. tumefaciens* nor *B. thuringiensis* is considered to be prevalent in the main receiving environment, that is the gastrointestinal tract of humans or animals. Both occur in soil, and, in addition, *B. thuringiensis* has been frequently isolated from the guts of insects (Jensen et al. 2003). However, occurrence of the recombinant genes outside their immediate receiving environment in the habitats of both bacterial species cannot be ruled out (Hart et al. 2009) and is therefore also considered here.

On a theoretical basis (i.e. without any study providing experimental evidence for horizontal gene transfer in the case of GM food and feed derived from soybean MON 87701 × MON 89788 or any other GM plant) it can be assumed that, as an extremely rare event, homologous recombination may occur in the environment between the recombinant *cry1Ac* or CP4 *epsps* genes and their natural variants as they may occur in *B. thuringiensis* (for *cry1Ac*) and *A. tumefaciens* (for CP4 *epsps*). 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 a *cry1Ac* or CP4 *epsps* gene would substitute genes for crown gall formation (loss of auxin-, cytokinin- and opine-synthesising genes). This event 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 theoretically possible. However, the transformation rates for illegitimate recombination are considered to be 10^{10}-fold lower

[30] Technical dossier/section D6

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 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.Both protein-encoding genes from bacteria are regulated in soybean MON 87701 × MON 89788 by promoters optimised for expression in plants: the *cry1Ac* gene by a promoter derived from the *A. thaliana RbcS4* gene and the CP4 *epsps* gene by a synthetic promoter derived from the promoter of the figwort mosaic virus and the *A. thaliana Tsf1* gene. The expression of the *prRBCS4–cry1ac* and P-*FMV/Tsf1*–CP4 *epsps* constructs 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, an *A. tumefaciens* recipient would become capable of producing an insecticidal Cry1Ac protein or a plant codon-optimised CP4 EPSPS protein. However, the exposure of bacterial communities to the recombinant genes in soybean MON 87701 × MON 89788 must 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 microorganisms occurring in the receiving environments. Owing to its specific lifestyle as a soil bacterium and plant pathogen, in contrast to the lifestyle of *B. thuringiensis*, which colonizes insect guts and infects specific target insects, the EFSA GMO Panel considers it unlikely that *A. tumefaciens* would gain selective advantage from such horizontal gene transfer by double homologous recombination.The EFSA GMO Panel concludes that the *cry1Ac* or CP4 *epsps* genes from soybean MON 87701 × MON 89788 may, on a theoretical basis, be transferred by double homologous recombination to *A. tumefaciens*. This event is highly limited by the short lengths of the DNA-flanking regions providing DNA homologies and also by the fact that *A. tumefaciens* is not a gut bacterium and thus not a member of the microbial community in the main receiving environment. Owing to the natural occurrence of *cry1Ac* and CP4 *epsps* in the environment, a low-level gene transfer to *A. tumefaciens* (for CP4 *epsps* and *cry1Ac*) or *B. thuringiensis* (for *cry1Ac*) is not regarded as conferring a new trait and selective advantage. Considering its intended use as food and feed and the above assessment, in agreement with its previous opinions on the single events, the EFSA GMO Panel has therefore not identified any concern associated with horizontal gene transfer from soybean MON 87701 × MON 89788 to bacteria.*(b) Plant-to plant-gene transfer*

Considering the intended uses of soybean MON 87701 × MON 89788 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 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*, *G. max* can cross only with other members of *Glycine* subgenus *Soja* (Hymowitz et al. 1998; Lu 2004). 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 Australia, China, Japan, Korea, the Philippines, the far eastern region of Russia, the South Pacific and Taiwan, 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 soybean in the EU.

Soybean is an annual mostly self-pollinating plant which has a percentage of cross-pollination in field crops 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 a 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 scenarios: imports of soybean MON 87701 × MON 89788 seeds (although most soybean MON 87701 × MON 89788 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 mainly 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 likelihood of all these conditions occurring and thereby resulting in cross-pollination between GM soybean plants and cultivated soybean is extremely low. Apart from seed production areas, GM plants and plants derived from outcrossing 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 (e.g. during transport 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 because of predation, rotting, germination resulting in death, or management practices prior to planting the subsequent crop (Owen 2005).

The EFSA GMO Panel takes into account the fact that this application does not include cultivation of the soybean within the EU, so that likelihood of cross-pollination between cultivated soybean and occasional soybean plants resulting from seed spillage is considered to be 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, as soybean MON 87701 × MON 89788 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 × MON 89788 in Europe will not differ from that of conventional soybean varieties.

6.1.2.3. Potential interactions of the GM plant with target organisms[31]

Owing to the intended uses of soybean MON 87701 × MON 89788, which exclude cultivation, and 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.

6.1.2.4. Potential interactions of the GM plant with non-target organisms[32]

Owing to the intended uses of soybean MON 87701 × MON 89788, 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.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 small quantity 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) and Cry1Ab/Ac fusion protein (Xu et al. 2009). There would, subsequently, be further degradation of the protein in the manure and faeces as a result of microbiological proteolytic activity.In addition, there will be further degradation of Cry proteins in soil, reducing the possibility for the exposure of potentially sensitive non-target organisms. While Cry proteins may bind to clay minerals and humic substances in soil, thereby reducing their availability to microorganisms for degradation, there are no indications of persistence and accumulation of Cry proteins from GM crops in soil (reviewed by Icoz and Stotzky 2008). The EFSA GMO Panel is not aware of evidence of released Cry proteins from GM plants causing significant negative effects on soil micro- or macroorganisms. Considering the scope of the application, it can be concluded that the exposure of

[31] Technical dossier/sections D8 and D9.4.
[32] Technical dossier/section D9.5.

potentially sensitive non-target organisms to the Cry1Ac protein is likely to be very low and of no biological relevance.

6.1.2.5. Potential interactions with the abiotic environment and biogeochemical cycles[33]

Owing to the intended uses of soybean MON 87701 × MON 89788, 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.

6.1.3. Post-market environmental monitoring[34]

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 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 2011b). The potential exposure to the environment of soybean MON 87701 × MON 89788 would be through manure and faeces from animals fed soybean MON 87701 × MON 89788 or through accidental release into the environment of GM soybean seeds (e.g. during transport and processing). The scope of the monitoring plan provided by the applicant is in line with the intended uses. 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 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 × MON 89788 as the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. 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 87701 × MON 89788. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

6.2. Conclusion

The scope of the application is for food and feed uses, import and processing of soybean MON 87701 × MON 89788 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 × MON 89788 and with the accidental release into the environment of viable seeds of soybean MON 87701 × MON 89788 (e.g. during transport and processing).In the case of accidental release into the environment of viable seeds of soybean MON 87701 × MON 89788 there are no indications of an increased likelihood of establishment and spread of feral soybean MON 87701 × MON 89788 plants, except under conditions of infestation of specific lepidopteran pests or the 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 87701 × MON 89788 to environmental bacteria does not raise concern owing to the lack of a selective advantage

[33] Technical dossier/sections D9.8 and D10.
[34] Technical dossier/section D11.

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 × MON 89788 and the guidance document of the EFSA GMO Panel on post-market environmental monitoring of GM plants (EFSA 2011b). In addition the EFSA GMO Panel acknowledges the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure in cases of accidental release of viable seeds of soybean MON 87701 × MON 89788. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The EFSA GMO Panel was requested to carry out an evaluation of a scientific risk assessment of the soybean MON 87701 × MON 89788 for food and feed uses, import and processing in accordance with Regulation (EC) No 1829/2003. The EFSA GMO Panel evaluated MON 87701 × MON 89788, which has been produced by conventional crossing of soybean lines containing the single events MON 87701 and MON 89788. Both single events have already been evaluated by the EFSA GMO Panel (EFSA 2008 2011a). In evaluating soybean MON 87701 × MON 89788 the EFSA GMO Panel considered the application EFSA-GMO-NL-2009-73, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications. Further information from applications for placing the single soybean events MON 87701 and MON 89788 on the market under the EU regulatory framework was taken into account.

The EFSA GMO Panel is of the opinion that the molecular characterisation data provided for soybean MON 87701 × MON 89788 are sufficient. The results of the bioinformatic analyses of the inserted DNA and the flanking regions of the single events MON 87701 and MON 89788 do not raise a safety concern. The levels of Cry1Ac and CP4 EPSPS proteins in soybean MON 87701 × MON 89788 have been sufficiently analysed, 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.

Previous evaluations of the single soybean events MON 87701 and MON 89788 showed that they do not differ in composition or agronomically and phenotypically from their corresponding conventional counterparts, except for the introduced traits and soybean MON 87701 having an increased vitamin E content. Both single events were within the ranges of commercial non-GM soybean varieties, except for the introduced traits. The results of the comparative analysis indicated that no biologically relevant differences were identified in the composition or phenotypic and agronomic characteristics of soybean MON 87701 × MON 89788 and its comparator soybean A5547, and that its composition fell within the range of non-GM soybean varieties, except for the presence of the newly expressed CP4 EPSPS and Cry1Ac proteins. A small increase in final stand count in soybean MON 87701 × MON 89788 was observed, but no safety issues were identified linked to this increase. Based on the assessment of the data available, the EFSA GMO Panel is of the opinion that crossing soybean MON 87701 and soybean MON 89788 to produce soybean MON 87701 × MON 89788 does not result in interactions that cause changes in composition or agronomic or phenotypic characteristics that would raise a safety concern.

The safety of the Cry1Ac protein expressed in MON 87701 and the CP4 EPSPS protein expressed in MON 89788 has been assessed previously, and no safety concerns were identified for humans and animals. In addition, the EFSA GMO Panel considers that it is unlikely that the overall toxicity and allergenicity of the whole soybean MON 87701 × MON 89788 has been changed. A feeding study with broiler chickens confirmed that the nutritional properties of soybean meal obtained from soybean MON 87701 × MON 89788 are not different from those of soybean meal from commercial non-GM soybean varieties. Potential interactions between the soybean events with respect to an effect on human and animal health were the focus of the assessment on food/feed issues. On the basis of the known functional characteristics and modes of action of the newly expressed proteins (Cry1Ac and CP4 EPSPS), the EFSA GMO Panel considers it unlikely that interactions between these proteins would occur that would raise any safety concerns. Thus, the EFSA GMO Panel concludes that soybean MON 87701 × MON 89788 is as safe and as nutritious as its comparator and commercial soybean varieties in the context of its intended uses.

Considering the intended uses of soybean MON 87701 × MON 89788, which exclude cultivation, there is no requirement for scientific assessment on possible environmental effects associated with the cultivation of this GM soybean. In the case of accidental release into the environment of viable seeds of soybean MON 87701 × MON 89788 (e.g. during transport and processing), there are no indications of an increased likelihood of establishment and spread of feral soybean plants, except under conditions of infestation of the specific lepidopteran pests or 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 87701 × MON 89788 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 87701 × MON 89788 and the guidance document of the EFSA GMO Panel on post-market environmental monitoring of GM plants (EFSA 2011b). 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 87701 × MON 89788.

In conclusion, the EFSA GMO Panel considers that the information available for soybean MON 87701 × MON 89788 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 × MON 89788 is as safe as its comparator with respect to potential effects on human and animal health or the environment in the context of its intended uses. In addition, the EFSA GMO Panel is of the opinion that crossing of single soybean events MON 87701 and MON 89788 to produce soybean MON 87701 × MON 89788 does not result in interactions between the events that would affect the safety of soybean MON 87701 × MON 89788 with respect to potential effects on human and animal health and on the environment, in the context of its intended uses.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of the Netherlands, received 1 September 2009, concerning a request for placing on the market soybean MON 87701 × MON 89788, submitted by Monsanto under Regulation (EC) No 1829/2003.

2. Acknowledgement letter, dated 17 September, from EFSA to the Competent Authority of the Netherlands (Ref. CGL/PB/KL/mt (2009) 4258912).

3. Letter from EFSA to applicant, dated 8 December 2009, delivering the 'Statement of Validity' for application EFSA-GMO-NL-2009-73 soybean MON 87701 × MON 89788, submitted by Monsanto under Regulation (EC) No 1829/2003 (Ref. PB/CE/lg (2009) 4497410).

4. Letter from EFSA to applicant, dated 26 February 2010, requesting additional information and stopping the clock (Ref. PB/KL/JA/lg (2010) 4675210).

5. Letter from applicant to EFSA, received 9 April 2010, providing additional information.

6. Letter from EFSA to applicant, dated 8 July 2010, requesting additional information and maintaining the clock stopped (Ref. PB/KL/lg (2010) 4982465).

7. Letter from EFSA to applicant, dated 8 July 2010, restarting the clock (Ref. PB/KL/CE/mt (2011) 5866844).

8. Letter from EFSA to applicant, dated 26 August 2011, requesting additional information and maintaining the clock stopped (Ref. EW/JA/AG/mt (2011) 5932927).

9. Letter from applicant to EFSA, received 5 September 2011, providing additional information.

10. Letter from EFSA to applicant, dated 13 October 2011, requesting additional information and maintaining the clock stopped (Ref. EW/ZD/JA/AG/mt (2011) 6025366).

11. Letter from applicant to EFSA, received 3 November 2011, providing additional information.

12. Letter from EFSA to applicant, dated 2 December 2011, requesting additional information and maintaining the clock stopped (Ref. EW/ZD/JA/AG/shv (2011) 6106761).

13. Letter from applicant to EFSA, received 6 December 2011, providing additional information.

14. Letter from EFSA to applicant, dated 20 January 2012, restarting the clock (Ref. EW/ZD/AG/shv (2012) 6190466).

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