SCIENTIFIC OPINION

Scientific Opinion on application (EFSA-GMO-NL-2008-52) for the placing on the market of herbicide tolerant genetically modified soybean A5547-127 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience[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 the genetically modified herbicide tolerant soybean A5547-127 for food and feed uses, import and processing. Soybean A5547-127 was developed through particle bombardment. It contains a single insertion site consisting of a copy of the intact pat expression cassette, encoding the PAT protein that confers tolerance to glufosinate-ammonium containing herbicides. Other inserted sequences include two truncated parts of the beta-lactamase (bla) gene from the transformation vector on each side of the pat expression cassette. The stability of the inserted DNA was confirmed over multiple generations. The results of the bioinformatic analyses of the insert and the flanking regions, and the levels of newly expressed protein did not raise a safety concern. The comparative analysis of compositional, phenotypic and agronomic characteristics indicated that soybean A5547-127 is not different from its conventional counterpart (A5547), except for the newly expressed protein (PAT). The safety assessment of the PAT protein and the soybean A5547-127 identified no concerns regarding potential toxicity and allergenicity. A feeding study on broiler chickens confirmed that seeds of soybean A5547-127 are as nutritional as seeds of the conventional counterpart. There are no indications of an increased likelihood of establishment and spread of feral soybean plants, except in the presence of the glufosinate-ammonium containing herbicides. The risk caused by a possible transfer of the recombinant gene from soybean A5547-127 to environmental micro-organisms is regarded to be negligible due to the lack of any advantage that would be conferred in the context of its intended uses. The potential interactions of the GM plant with target organisms, non-target organisms and the abiotic environment and biogeochemical cycles were not considered an issue by the EFSA GMO Panel. The monitoring plan and reporting intervals are in line with the intended uses of soybean A5547-127. The EFSA GMO Panel considers that the

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information available for soybean A5547-127 addresses the scientific comments raised by the Member States and that the soybean A5547-127, as described in this application, is as safe as its conventional counterpart with respect to potential effects on human and animal health and the environment in the context of its intended uses.

KEY WORDS

GMO, soybean (*Glycine max*), A5547-127, herbicide tolerance, glufosinate-ammonium containing herbicides, *pat* gene, PAT protein, human and animal health, import and processing, Regulation (EC) No 1829/2003

SUMMARY

Following the submission of an application (EFSA-GMO-NL-2008-52) under Regulation (EC) No 1829/2003 from Bayer CropScience, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) was asked to deliver a scientific opinion on the safety of herbicide tolerant genetically modified (GM) soybean A5547-127 (Unique Identifier ACS-GM∅∅6-4) for food and feed uses, import and processing.

In delivering its scientific opinion, the EFSA GMO Panel considered the application EFSA-GMO-NL-2008-52, additional information supplied by the applicant, scientific comments submitted by the Member States, and relevant scientific publications. The scope of application EFSA-GMO-NL-2008-52 is for food and feed uses, import and processing of soybean A5547-127 within the European Union as any non-GM soybean but excludes cultivation in the EU. The EFSA GMO Panel evaluated soybean A5547-127 with reference to the intended uses and appropriate principles described in its Guidance Document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed (EFSA, 2006a). The scientific evaluation of the risk assessment included molecular characterisation of the inserted DNA and expression of the corresponding proteins. An evaluation of the comparative analysis of composition, phenotypic and agronomic characteristics was undertaken, and the safety of the new proteins and the whole food/feed was evaluated with respect to potential toxicity, allergenicity and nutritional wholesomeness. An evaluation of the environmental impacts and the post-market environmental monitoring plan were undertaken.

Soybean A5547-127 was transformed using particle bombardment. Soybean A5547-127 expresses the *pat* gene leading to the production of the enzyme phosphinothricin acetyl-transferase (PAT) that acetylates L-glufosinate. The PAT enzyme confers tolerance to glufosinate-ammonium containing herbicides.

The molecular characterisation data establish that the genetically modified soybean A5547-127 contains one copy of an intact *pat* expression cassette in a single insertion locus. Other parts of the plasmid used for transformation and present in soybean A5547-127, include two truncated, non-functional parts of the beta-lactamase (*bla*) gene on each side of the *pat* expression cassette. Bioinformatic analysis of the open reading frames spanning the junctions created as results of the transformation did not raise safety concerns. The stability of the inserted DNA was confirmed over several generations. Analysis of the levels of the PAT protein in seed from a field trial performed in the USA did not raise safety concerns.

Based on the results of a comparative analysis of data, the EFSA GMO Panel concludes that from the compositional point of view, the differences observed between the soybean A5547-127 and the conventional counterpart were not consistent across sites and years and fell within the range of natural variation of soybean. From the phenotypic and agronomic points of view, the EFSA GMO Panel concludes that soybean A5547-127 is phenotypically and agronomically not different from its conventional counterpart A5547, with the exception of the newly introduced trait.The PAT protein is quickly degraded in simulated gastric and intestinal fluids without leaving stable peptide fragments. Bioinformatics-supported studies demonstrated that the PAT protein shows no homology to known toxic and allergenic proteins. It also induced no toxicity when administered orally to mice in a repeated dose toxicity study. Testing the reaction of sera from soybean-allergic patients to extracts from soybeans A5547-127 and A5547 and commercial soybean varieties demonstrated that the overall allergenicity of soybean A5547-127 is not different from that of the conventional counterpart and commercial soybean varieties. A feeding study on broiler chickens confirmed that soybean A5547-127 is as nutritional as soybean A5547. The EFSA GMO Panel is of the opinion that soybean A5547-127 is as safe as its conventional counterpart and commercial varieties based on data from literature.

The application EFSA-GMO-NL-2008-52 is for food and feed uses, import and processing. Therefore, there is no requirement for scientific information of possible environmental effects associated with the cultivation of soybean A5547-127. There are no indications of an increased likelihood of establishment and spread of feral soybean plants in case of accidental release into the environment of viable seeds of soybean A5547-127 (e.g.; during transportation and processing), except in the presence of glufosinate-ammonium containing herbicides. Taking into account the scope of the application, the rare occurrence of feral soybean plants and the low levels of exposure through other routes, the risk to non-target organisms is extremely low. The risk caused by a possible transfer of the recombinant gene from soybean A5547-127 to environmental micro-organisms is regarded to be negligible due to the lack of any advantage that would be conferred in the context of its intended uses. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan. The EFSA GMO Panel recommends that appropriate management systems should be in place to restrict seeds of soybean A5547-127 entering cultivation as the latter requires specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

In conclusion, the EFSA GMO Panel considers that the information available for soybean A5547-127 addresses the scientific comments raised by the Member States and that the soybean A5547-127, as described in this application, is as safe as its conventional counterpart with respect to potential effects on human and animal health and the environment in the context of its intended uses.

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BACKGROUND

On 3 April 2008, the European Food Safety Authority received from the Dutch Competent Authority an application (Reference EFSA-GMO-NL-2008-52) for authorisation of genetically modified (GM) soybean A5547-127 (Unique Identifier ACS-GMØØ6-4) submitted by Bayer CropScience within the framework of Regulation (EC) No 1829/2003 on GM food and feed (EC, 2003). After receiving the application EFSA-GMO-NL-2008-52 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 5 June and 26 June 2008, EFSA received additional information requested under completeness check (requested on 30 May 2008 and 13 June 2008). On 18 July 2008, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the European Commission and consulted nominated risk assessment bodies of the Member States, including the national Competent Authorities within the meaning of Directive 2001/18/EC (EC, 2001) 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 28 October 2008) within which to make their opinion known.

The Scientific Panel on Genetically Modified Organisms of EFSA (EFSA GMO Panel) carried out an evaluation of the scientific risk assessment of the GM soybean A5547-127 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. The EFSA GMO Panel carried out the safety evaluation in accordance with the appropriate principles described in the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006a). In addition, the scientific comments of the Member States, the additional information provided by the applicant, and relevant scientific publications were taken into consideration.

On 05 December 2008 and 29 June 2009, the EFSA GMO Panel requested additional information from the applicant. The applicant provided the requested information on 16 April 2009 and 09 December 2010.

In giving its opinion on soybean A5547-127 to the European Commission, the Member States and the applicant, and in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003, EFSA has endeavoured to respect a time limit of six months from the acknowledgement of the valid application. As additional information was requested by the EFSA GMO Panel, the time-limit of 6 months was extended accordingly, in line with Articles 6(1), 6(2), 18(1), and 18(2) of Regulation (EC) No 1829/2003.

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

TERMS OF REFERENCE

The EFSA GMO Panel was requested to carry out a scientific assessment of soybean A5547-127 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. Where applicable, any conditions or restrictions which should be imposed on the placing on the market and/or specific conditions or restrictions for use and handling, including post-market monitoring requirements based on the outcome of the risk assessment and, in the case of GMOs or food/feed containing or consisting of GMOs, conditions for the protection of particular

ecosystems/environment and/or geographical areas should be indicated in accordance with Articles 6(5)(e) and 18(5)e of Regulation (EC) No 1829/2003.

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

ASSESSMENT

1. Introduction

The genetically modified (GM) soybean A5547-127 (Unique Identifier ACS-GMØØ6-4) was evaluated with reference to its intended uses, taking account of the appropriate principles described in the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006a). The evaluation of the risk assessment presented here is based on the information provided in the application, as well as additional information from the applicant, scientific comments submitted by the Member States and relevant scientific publications.

2. Issues raised by Member States

The issues raised by the Member States are addressed in Annex G of the EFSA overall opinion[4] and have been considered in this scientific opinion.

3. Molecular characterisation

3.1. Evaluation of relevant scientific data

3.1.1. Transformation process and vector constructs[5]

Soybean tissue from embryo shoot apices derived from surface-sterilized seeds was transformed with *Pvu*I-digested DNA of the plasmid vector pB2/35SAcK using particle bombardment. The vector is derived from pUC19 and consists of the complete plasmid with, among others, the ColE1 origin of replication, the *bla* gene conferring resistance to ampicillin in bacteria, a synthetic right border (RB) identical to the *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*) Ti plasmid pTiAch5 and the *pat* expression cassette. Prior to transformation, the pB2/35SAcK vector was digested with *Pvu*I to disrupt the *bla* gene coding region, generating two fragments, one (3119 bp) carrying the *pat* expression cassette, the ColE1 origin of replication and the 3' part of the *bla* gene and the other (957 bp) carrying the 5' part of the *bla* gene and the RB border element. The digested pB2/35SAcK DNA was used for the transformation.

The *pat* expression cassette consists of the following elements: the 35S promoter from *Cauliflower mosaic virus* (CaMV), a *pat* gene to confer tolerance to glufosinate and glufosinate-ammonium containing herbicides, and the 35S transcription terminator from CaMV. The *pat* gene is a synthetic version of the *Streptomyces viridochromogenes* gene with a modified DNA sequence for optimal expression in plants. A *Sal*I site preceding the ATG start codon of the *pat* gene was removed. These modifications in the DNA sequence do not alter the predicted amino acid sequence of the PAT protein.

3.1.2. Transgene constructs in the genetically modified plant[6]

Southern analysis of genomic DNA with eight restriction enzymes and a combination of two of them was performed using four probes that cover the inserted *pat* gene cassette and the *bla* sequences (*pat*, 3' *bla*, 5' *bla* + vector, entire cassette). This analysis demonstrated that the insert consists of one copy of the *pat* expression cassette and the ColE1 origin of replication as well as two truncated parts of the

[4] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2011-00292
[5] Technical Dossier / Sections C and D1
[6] Technical Dossier / Section D2

bla gene one on each side of the cassette, all at a single locus. Additional Southern analyses of genomic DNA digested with five different restriction endonucleases using probes covering the rest of the vector used for transformation confirmed the absence of additional insertions.

The soybean A5547-127 event was further characterized by determining the sequence of two PCR-amplified overlapping fragments representing the complete insert. The two fragments of the *bla* gene located at the 5' and 3' flanking regions consist of nucleotides from 419 to 29, and from 420 to 861, respectively. The two *bla* fragments are integrated in the same orientation, but in reverse orientation with respect to the *pat* expression cassette. The two *bla* fragments do not constitute a functional gene. The sequence of the insert is identical to the corresponding regions of the plasmid used for transformation.

The sequence of the preinsertion locus was also determined. Alignment of the sequence of the preinsertion locus with the 3' and 5' flanking sequences of soybean event A5547-127 confirmed that the insertion occurred in the soybean genomic DNA and revealed no additional insertions or deletions at the integration site. BLASTN analysis of 1553 bp sequence of the preinsertion locus revealed similarity with transcribed sequences of genes to which no function has been assigned. Compositional, phenotypic and agronomic analyses showed that soybean A5547-127 is not different from its conventional counterpart (sections 4.1.3, 4.1.4) except for the expected trait, indicating that the insertion of the transgene has not altered the expression of an essential gene and the insertion of the transgene at this site does not raise a safety concern.

During the transformation three new DNA junctions were created: two between the insert and the soybean genomic DNA and one inside the insert. Bioinformatic analyses were performed to identify open reading frames spanning the junction regions. The deduced amino acid sequences of all the predicted open reading frames were compared to sequences of known toxins and allergens, by using BLASTP or FindPatterns algorithms. No significant similarities with known toxins or allergens were found.

3.1.3. Information on the expression of the insert[7]

The level of PAT protein was measured by ELISA in soybean A5547-127 samples cultivated in Florida, USA (1999) and under greenhouse conditions (2007). The expression levels were determined from seeds (field trial) and from leaf, stem and root at two growth stages (greenhouse trial). Considering the scope of the application, the protein expression data related to the seeds are considered the most relevant. PAT protein was present in the seed at 17.5 µg/g and 20.2 µg/g fresh weight in plants sprayed and non-sprayed with glufosinate-ammonium containing herbicides, respectively.

Analyses were performed to determine whether the *bla* gene fragments were expressed in soybean event A5547-127. Northern analysis of total RNA extracted from leaf, stem and root tissue as well as from seeds gave no indication that the 3' or 5' truncated *bla* fragments are transcribed in any of the tested tissues. In the unlikely event that transcription did occur, a functional protein would not be produced.

3.1.4. Inheritance and stability of the inserted DNA[8]

To demonstrate the stability of the soybean A5547-127 event Southern analysis using a probe covering the *pat* gene cassette was performed on DNA from plants produced over three successive generations (R3, R4, R5) digested with two different restriction endonucleases. The analysis showed

[7] Technical Dossier / Section D3
[8] Technical Dossier / Section D5

that the overall structure of the insert and the adjacent plant DNA was conserved in the three generations. These results indicate the stability of the soybean event at the genomic level over multiple generations.

3.2. Conclusion

Appropriate molecular and bioinformatic analyses of the soybean A5547-127 insert and its flanking genomic regions have been undertaken. The expression of the introduced gene has been sufficiently analysed and the stability of the genetic modification has been demonstrated over several generations. The molecular characterisation provided for the transformation event soybean A5547-127 is sufficient for the safety assessment and does not indicate a safety concern.

4. Comparative analysis

4.1. Evaluation of relevant scientific data

4.1.1. Choice of comparator and production of material for the compositional assessment[9]

In the application EFSA-GMO-NL-2008-52 for food and feed use, import and processing of soybean A5547-127 within the European Union, the applicant presented compositional data of seed material of soybean A5547-127 and soybean A5547 collected in field trials in the USA (North Carolina, Florida, Georgia, Mississippi, Arkansas, Louisiana and Texas) in 1999 (4 sites), 2000 (5 sites), 2006 (7 sites) and 2007 (8 sites) (Shillito, 2001a, 2001b; Kowite, 2007; Oberdörfer, 2008; Rattemeyer-Matschurat, 2008a). The Asgrow variety A5547 was the conventional soybean long-season variety (type V) used when the soybean was transformed to establish transformation event A5547-127, and thus has a comparable genetic background to A5547-127. The field trial sites selected for the comparative studies were typical regions for growing type V soybeans in the USA. At each field trial site, soybean A5547-127 and the conventional counterpart (A5547) were planted following a randomized complete block design with three replicates per site. The conventional counterpart was compared with soybean A5547-127 not treated with glufosinate-ammonium containing herbicides and soybean A5547-127 treated two times with this type of herbicide. The plant materials collected from the field trials in 1999, 2000 and 2006 were used for the compositional comparison, and field trials in 2006 and 2007 used for the phenotypic and agronomic comparisons between soybean A5547-127 and its conventional counterpart. Three of the field trial sites in 1999 and 2000 as well as in 2000 and 2006 were comparable and allowed a by year analysis of obtained data.

4.1.2. Compositional analysis[10]

Soybean seeds were analysed for proximates and fiber (compounds) fractions, as well as for amino acids, fatty acids, minerals, vitamins, anti-nutrients (i.e. phytic acid, trypsin inhibitors, lectins, stachyose and raffinose) and some other metabolites (isoflavones). In total 84 different compounds were analysed, including those recommended by OECD (2001). The comparison was done between levels in soybean A5547-127 and soybean A5547 within-field trial site and across-sites (data from all sites combined) each year of field trials. Several of the fatty acids analysed were rare and occurred at levels below the limit of quantification. When all samples for a treatment at a field trial site were below the limit of quantification the applicant considered the respective samples/treatments to be not significantly different.

[9] Technical dossier/ Section D7.1 and D7.2
[10] Technical dossier/ Section D7.2 and D7.3

The compositional comparison performed within sites for any of the three growing seasons 1999, 2000 and 2006 showed quite a high number of statistically significant differences both between the conventional counterpart and soybean A5547-127 non-sprayed with glufosinate-ammonium and between the conventional counterpart and soybean A5547-127 sprayed with this herbicide (Shillito, 2001a, 2001b; Kowite, 2007; Oberdörfer, 2008; Rattemeyer-Matschurat, 2008a). The level of two of the 84 compounds studied, oleic acid and raffinose, showed a statistically significant difference between soybean A5547-127 and the conventional counterpart in materials from the majority of the field trial sites (9 of 16 sites in both cases). When the statistical evaluation was focused on the data from field trial sites that were comparable between years, content of eight of the 84 compounds analysed were statistically significant from the control in both the non-sprayed and glufosinate-ammonium sprayed soybean A5547-127. These compounds were palmitic acid (16:0), stearic acid (18:0), calcium, phosphorous, vitamin B1, phytic acid, raffinose and stachyose. However, all differences, including that of oleic acid, were small. Regarding palmitic acid and stearic acid, the difference between soybean A5547-127 and its comparator was not consistent, being an increase one year and a decrease the other two years. Whereas levels of raffinose and stachyose were reduced by around 5% and 7% in soybean A5547-127 as compared to soybean A5547, the levels of calcium, phosphorous, vitamin B1 and phytic acid were slightly increased (around 6%, 4%, 7%, and 3%, respectively). Although statistically significant differences were found, they were not consistently observed in seed materials from the field trials and in sprayed and non-sprayed samples, and were always of a low magnitude and within the normal range observed in commercial varieties of non-GM soybean based on data from scientific literature. None of the differences were considered to have nutritional or toxicological implications.

Soybeans contain a number of isoflavone compounds, reported to have both beneficial and adverse biological effects at higher intake levels. In the plant, they occur as glucosides, acetylglucosides, or malonylglucosides, but they are commonly reported as aglycones. Whereas no statistically significant difference between soybean A5547-127 (sprayed or non-sprayed with glufosinate-ammonium containing herbicides) and the conventional counterpart was found for glucosides, levels of the daidzein, genistein, and glycitein aglycone equivalents were reduced in soybean A5547-127. However, the levels found were in all cases within the natural variation of these constituents in soybean described in the USDA-ISU (2006) isoflavone database and in the literature (OECD, 2001; ILSI, 2007).

The EFSA GMO Panel considered the total set of compositional data supplied and the observed compositional differences between soybean A5547-127 and its conventional counterpart in the light of the field trial design, biological variation and the level of the studied compounds reported in conventional soybean varieties in the scientific literature, and concludes that the differences observed between soybean A5547-127 and the conventional counterpart (A5547) were not consistent across sites and years and fell within the range of natural variation of soybean.

4.1.3. Agronomic traits and GM phenotype[11]

The applicant provided information on agronomic performance, phenotypic characteristics and ecological interaction of soybean A5547-127 (both unsprayed and sprayed with glufosinate-ammonium containing herbicides) and soybean A5547 (control unsprayed with glufosinate-ammonium containing herbicides) from a large number of field trials (Van Wert, 1998; Kowite, 2007, 2008). Comparative field observations of soybean A5547-127 and A5547 plants were performed in the United States at 19 field trial sites in 1996, 48 sites in 1997, 8 sites in 2006 and 8 sites in 2007. The field trials in the 1990's were made as part of the event evaluation process and included

[11] Technical dossier/ Section D7.4

evaluation of characteristics important for varietal registration, whereas the two latter years of studies focused on morphological and agronomic parameters. Traits evaluated in these field trials included both qualitative and quantitative characteristics: emergence, stand count, plant vigour and health rating, days to 50% flowering (flowering date), flower morphology and colour, pubescence colour, pod colour, hilum colour and shape, canopy architecture, leaf shape, plant height, susceptibility to pests and diseases, pollen viability and germination, and yield.

The statistical analysis over all sites revealed no statistically significant differences in the quantitative traits 50% plant emergence, stand count, plant vigour and yield (Rattemeyer-Matschurat, 2008b). There was also no difference in flowering date, plant height and days to maturity between soybean A5547-127 and A5547 at the majority of the field trial sites (Rattemeyer-Matschurat, 2008b). Similarly no overall difference between these two soybean varieties was found in seven qualitative characteristics (flower colour, pubescence colour, pod colour, hilum colour, canopy architecture, leaf shape and susceptibility to pests and diseases). With the exception of stand count, the results were the same when the control soybean was compared to soybean A5547-127 sprayed with glufosinate as it was when compared to soybean A5547-127 non-sprayed with this herbicide. For stand count a statistical difference between the soybean A5547-127 and its comparator was observed when GM soybean was sprayed with glufosinate-ammonium containing herbicides (Rattemeyer-Matschurat, 2008b). However, the difference was noted at only two of the sixteen field trial sites studied. Thus, regarding the morphological and agronomic data significant differences between treatments were sometimes observed at individual field trial sites but when the entire material was considered no unintended alteration could be identified.

The EFSA GMO Panel assessed the provided data and considers soybean A5547-127 to be phenotypically and agronomically not different from its conventional counterpart A5547, with the exception of the newly introduced trait.

4.2. Conclusion

Based on the results of a comparative analysis of data, the EFSA GMO Panel concludes that from the compositional point of view, the differences observed between the soybean A5547-127 and the conventional counterpart were not consistent across sites and years and fell within the range of natural variation of soybean. From the phenotypic and agronomic points of view, the EFSA GMO Panel concludes that soybean A5547-127 is phenotypically and agronomically not different from its conventional counterpart A5547, with the exception of the newly introduced trait.

5. Food/feed safety assessment

5.1. Evaluation of relevant scientific data

5.1.1. Product description and intended uses[12]

The scope of application EFSA-GMO-NL-2008-52 is for food and feed uses, import and processing of soybean A5547-127 and its derived products within the European Union. Thus, soybean A5547-127 will be imported into the EU mixed with other soybean varieties and be used as food or feed, or for the production of a large number of derived products, as any commercial soybean variety. The main product for human use is soybean oil. Around 10% of the heat-processed (toasted) defatted soybean meal goes to production of soybean products for human consumption, including flours, soybean

[12] Technical Dossier/ Section A5-A7

protein concentrates and various textured products simulating meats, sea-foods and cheeses. These products are used to produce traditional soya foods such as miso, tofu, soya sauce and soymilk, and the emulsifying agent lecithin. The rest of the toasted defatted soybean meal goes to feed, in the European Union mainly to poultry, pig and cattle (OECD, 2001). Whole soybeans are used to produce soya sprouts, baked and roasted soybeans. There is also a limited direct use of soybeans as animal feeds.

The genetic modification of soybean A5547-127 results in the expression of the PAT protein, an enzyme that acetylates the active herbicide ingredient glufosinate-ammonium, thereby detoxifying the herbicide. The genetic modification, therefore, allows soybean A5547-127 to grow also in the presence of glufosinate-ammonium containing herbicides. Thus, the genetic modification is intended to improve agronomic performance only and is not intended to influence the nutritional aspects, the processing characteristics and overall use of soybean as a crop.

5.1.2. Effects of processing[13]

Soybean A5547-127 will be used for production and manufacturing of food and feed products as any other commercial soybean variety. Taking into account the compositional analysis (see Section 4.1.2), which gives no indication of relevant compositional changes as compared to the conventional counterpart, there is no reason to expect that the characteristics of soybean A5547-127 and derived processed products would be different from those of the respective products derived from conventional soybean varieties. To confirm this assumption, the applicant studied the effect of storage and processing on the PAT content of A5547-127 soybean seed (3993-9113 ng PAT protein/g; 0.001-0.002% of crude protein), straw (<80 ng PAT protein/g; 0.00009% of crude protein) and forage (2820-5736 ng PAT protein/g; 0.002-0.003% of the crude protein), and the influence of processing on the chemical composition of the derived products hulls, non toasted meal, toasted meal, crude oil, refined food grade oil, soybean isolate and lecithin of GM soybean A5547-127 as compared to the corresponding products of soybean A5547 (Scott, 2001; Shillito, 2001c, 2003). Storage of the mentioned materials in the freezer for two years had marginal effects on the PAT protein content (Scott, 2001). Before processing, the fresh seed samples of glufosinate-ammonium sprayed soybean A5547-127 on average contained 17 471 ng PAT/g fresh weight. On a fresh weight basis, hulls of glufosinate-ammonium sprayed soybeans on average contained 9 521 ng PAT protein/g sample. The corresponding figures for meal was 69.5 ng/g, toasted meal 13.4 ng/g, and soybean isolate 80.9 ng/g. Thus, processing reduced the PAT content considerably. Levels were below the limit of quantification (6.6 ng/g for oil and 4 ng/g for crude lecithin) for refined oil, refined bleached and deodorized oil and crude lecithin. The influence of the temperature on the isolated PAT protein was followed by SDS-PAGE and Coomassie blue staining. Although the enzymatic activity was suppressed already at 50^{o}C, temperatures up to 90^{o}C for up to 60 minutes resulted in no fragmentation of the PAT protein (Esdaile, 2002). Regarding other soybean constituents, taken together, these analyses showed that processing conditions sometimes reduces the amount of some chemical constituents but the reduction is comparable in the GM soybean A5547-127 and the conventional counterpart.

In addition to the compositional studies on soybean seeds presented in section 4.1.2, the applicant compared the composition of processed products of GM soybean A5547-127 (unsprayed and sprayed with glufosinate-ammonium containing herbicides) with that of the conventional counterpart. Products studied were hulls, untoasted defatted meal, toasted defatted meal, refined bleached and deodorized oil, and soya isolate. Although some data from products processed from both soybean A5547-127 and the conventional counterpart did not fall within the range of values reported in the literature, these latter data could have been obtained from products produced by other processing technology, the composition of the processed products did not raise safety concern.

[13] Technical Dossier/ Section D7.6

5.1.3. Toxicology[14]

5.1.3.1. Protein used for safety assessment

Due to the relatively low expression level of the PAT protein in soybean A5547-127 (see section 3.1.3) and the very difficult task to isolate a sufficient quantity of purified protein from the genetically modified soybean, the safety studies with the newly expressed protein were conducted with a PAT protein encoded by the *pat* gene from *Streptomyces viridochromogenes* and expressed in *Escherichia coli*. The structural similarity and physicochemical and functional equivalence of the PAT protein produced by *E. coli* to that produced in leaf material of soybean A5547-127 was demonstrated by N-terminal sequencing (Edman degradation), Western analysis with PAT specific antibodies, mobility in SDS-PAGE, analysis by HPLC/Electrospray mass spectrometry, glycosylation analysis and determination of PAT enzymatic activity. Together these methods confirmed the equivalence of the bacterial and the plant PAT proteins (Currier and Hendrickx, 2007). The only difference identified was that the protein isolated from soybean A5547-127 is missing the N-terminal methionine present in the *E. coli*-derived PAT protein. As shown by Bradshaw *et al*. (1998) post-translational modification of the type observed is not infrequent in proteins from both prokaryotic and eukaryotic organisms. Based on the identified similarity in structure, and equivalence in physico-chemistry and function between these proteins, the EFSA GMO Panel accepts the use of a PAT derived from *E. coli* for the degradation studies and safety testing of the PAT protein present in soybean A5547-127 and as a reference standard in the enzyme-linked immunosorbent assay (ELISA) to estimate PAT expression levels in various tissues of soybean A5547-127.

5.1.3.2. Toxicological assessment of the expressed novel protein in soybean A5547-127

The newly introduced gene in soybean A5547-127 is derived from the gram positive sporulating soil bacterium *Streptomyces viridochromogenes*. The *pat* gene codes for an enzyme, PAT, unknown to be toxic to humans and animals. Although this specific PAT protein does not occur in conventional food and feed, it belongs to the class of acetyltransferase enzymes common to plants and animals. In addition, PAT-expressing genetically modified plants have now been consumed as food and feed for over ten years without any adverse effects to human and animal health having been reported. A safety evaluation of PAT proteins encoded by the pat and bar sequences that confer tolerance to glufosinate-ammonium containing herbicides in transgenic plants has been published (Hérouet *et al*., 2005).

(a) Acute toxicity testing

The PAT protein produced in a recombinant *E. coli* strain did not induce adverse effects in an acute toxicity study in OF1 female mice administered a single dose of 0, 1 or 10 mg of the protein per kg body weight intravenously (Kennel, 2003; Hérouet, 2004).

(b) Repeated oral toxicity study

The applicant provided a 14-day repeated dose feeding study in which groups of 5 Wistar rats of each sex were given a low protein diet containing 0, 0.5 or 5.0% (w/w) of a lyophilized powder of the PAT protein (Pfister *et al*., 1999). The highest dietary inclusion resulted in a daily dose of ca. 7.6 and 7.9 g/kg body weight for males and females, respectively. The total protein levels in the diets for the control and low-dose groups were adjusted with soya protein from commercial non-GM soybeans to reach a level comparable to that in the diet for the high-dose group. An additional group was fed a standard rodent diet. There was no mortality, and no relevant influence on food consumption and body weight development induced by the treatments. At the end of the treatment period haematology, clinical chemistry and urine alysis were performed, organ weights were determined, and macroscopic

[14] Technical Dossier/ Section D7.8

and histopathology examinations of selected organs and tissues were carried out. It is concluded that the repeated dose toxicity study in rats gave no indications for adverse effects attributable to the PAT protein up to the highest dose tested.

(c) Degradation in simulated digestive fluids

Digestion of the PAT protein (183 amino acids) in a pepsin digestion assay (simulated gastric fluid) performed at pH 2.0 was studied *in vitro* by identifying the enzyme and peptide fragments using SDS-PAGE colloidal blue gel staining (Rouquie, 2005). The SDS-PAGE colloidal blue gel staining demonstrated that the PAT protein produced in *E. coli* was degraded within 30 seconds. Additional digestibility studies, using Western analysis, showed that the PAT protein is almost immediately degraded in simulated intestinal fluid containing pancreatin (pH 7.5). Residual fragments having a size of 5-14 kDa disappeared in less than 30 seconds using this assay (Esdaile, 2004).

The applicant also reported on the inactivation of a plant-derived PAT protein (source not defined) in gastric juice from pigs and cattle, using the enzymatic activity of PAT as an indicator (Schulz, 1993). These studies showed that the enzyme activity quickly declined after incubation of a crude protein extract in pig stomach fluid and bovine rennet-bag fluid at low pH (pH 1.3-5) and declined, but at a lesser rate, after incubation at higher pH (pH 5-7).

(d) Bioinformatics studies

Searches for amino acid sequence homology of the PAT protein expressed in soybean A5547-127 with amino acid sequences of toxic proteins stored in databases indicated significant homology only with other acetyl-transferase proteins. No sequence homology with known toxic proteins was found (Capt, 2007b, 2007c, 2007d). Thus, no safety concerns for humans and animals were identified.

5.1.3.3. Toxicological assessment of new constituents other than proteins

No new constituent other than the PAT protein is expressed in soybean A5547-127 and no relevant changes in the composition of soybean A5547-127 were detected in relation to the composition of soybean A5547.

5.1.3.4. Toxicological assessment of the whole GM food/feed

Although the insert in soybean A5547-127 disrupted a soybean gene to which no known function could be assigned (section 3.1.2), no indication was found in the molecular analysis and in the comparative compositional, phenotypic and agronomic analysis that this resulted in any unintended changes. Thus, GM soybean 5547-127 is compositionally and agronomically not different from its conventional counterpart and equivalent to commercial varieties based on data from literature. According to the guidance document of the EFSA GMO Panel (EFSA, 2006a), animal safety studies with the whole food/feed are therefore not required.

5.1.4. Allergenicity[15]

The strategies used when assessing the potential allergenic risk focus on the characterisation of the source of the recombinant protein, the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons and whether the transformation may have altered the allergenic properties of the modified food. A weight-of-evidence approach is recommended, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (CAC, 2003; EFSA, 2006a).

[15] Technical Dossier/ Section D7.9

5.1.4.1. Assessment of allergenicity of the newly expressed proteins

The *pat* gene originates from *Streptomyces viridochromogenes,* a soil micro-organism that is not known to give rise to allergenicity. The PAT protein is constitutively expressed in soybean A5547-127. The most relevant tissue for the assessment of food allergenicity is the seed, which was shown to contain around 17.5 µg/g seed fresh weight (0.0048% of crude protein) in sprayed soybean plants, and around 20.2 µg/g seed fresh weight (0.0056% of crude protein) in non-sprayed plants, respectively. Thus, the PAT protein occurs at relatively low levels in soybean tissues.

Bioinformatics-supported comparisons of the amino acid sequences of the PAT protein and potential proteins encoded by ORFs (within the inserted DNA) with amino acid sequences of known allergens available in general and allergen databases were performed (Capt, 2007a, 2007b, 2007c, 2007d). These analyses initially compared 80 amino acid long sequences of the tested proteins with equally long amino acid sequences of proteins available in general protein databases, using a BLASTP algorithm. Using a cut-off point of at least 35% amino acid identity, the PAT protein presented a high sequence similarity only with other acetyl-transferases not known to be allergenic. Further bioinformatics testing using 100% identity between sequences of 8 contiguous amino acids as criterion did not identify any matches to known allergens using the FindPatterns algorithm and an allergen data base on 1408 sequences build by the applicant from public reference databases. The *in silico* studies also identified no potential post-translational glycosylation sites on the PAT protein. Thus, no indication that the proteins resemble known allergens was found.

In addition, human exposure to the intact protein can be considered low as the PAT is not detected in soybean oil, is not stable in acidic environments, is rapidly degraded under simulated gastric and intestinal fluid conditions, and may, like other proteins, be inactivated during processing of soybeans. Based on the overall information, the EFSA GMO Panel considers it unlikely that the newly expressed PAT protein is an allergen.

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

Allergenicity of the whole crop could be increased as an unintended effect of the random insertion of the transgene in the genome of the recipient, for example through qualitative or quantitative modifications of the pattern of expression of endogenous proteins. However, given that equivalence to the conventional counterpart was demonstrated (with the exception of the introduced trait) on the basis of extensive compositional, phenotypic and agronomic analysis, no increased allergenicity is anticipated for soybean A5547-127.

Because the soybean is a recognised allergenic food, the applicant performed extensive *in vitro* allergenicity studies with extracts of soybeans A5547-127 and its conventional counterpart (A5547) using sera obtained from 16 American patients being allergic to soybean (Lehrer, 1997). The sera were tested for antibody reactivity in RAST (Radio AllergoSorbent Test), RAST inhibition and immunoblotting studies, and the result indicated no relevant difference in reactivity of the sera to the two soybean extracts. On request from the EFSA GMO Panel[16], the applicant supplied a detailed comparative analysis of the IgE 2D gel blotting patterns of endogenous allergens from soybean A5547-127, its conventional counterpart (A5547) and two commercial soybean varieties (Stine 2686-6 and Stine 2788). These studies showed that the IgE binding properties of sera from ten soybean allergic subjects and four non-soybean allergic controls were comparable (no relevant qualitative and quantitative changes) for extracts from soybean A5547-127 and its conventional counterpart. The EFSA GMO Panel concluded that the information provided confirms that the overall allergenicity of the whole plant is not changed.

[16] Additional information 9 December 2010

5.1.5. Nutritional assessment of GM food/feed[17]

The applicant reported 9.5-20.2 µg PAT protein/g fresh weight soybean seeds or hulls, and very much less in toasted soybean meal (Shillito, 2003). Thus, farm animals are exposed to low levels of the PAT protein via feed. Although not required as soybean A5547-127 was shown to be compositionally not different from its conventional counterpart, the applicant provided a 42-day broiler chicken feeding study using a non-defined commercial strain of broilers and two types of diets (Leeson, 1998). Each treatment consisted of 120 female broilers (6 replicates of 20 birds; replicates in a randomised complete block design). Whereas one group of chickens received diets with 20% seeds of heat-treated soybean A5547-127, another group received diets with 20% heat-treated seeds of the conventional counterpart. The diets also contained meal from conventional non-GM soybeans, in this case the amount differing between diets given early, in the middle or late in the study. Thus starter diets (day 1-17) contained 18.24%, grower diets (day 17-31) 14.94% and finisher diets (day 31-42) 11.69% soybean meal. The crude protein content decreased during the study from 22.0% during day 1-17, to 20.5% day 17-31, and 19.1% during day 31-42.

There was no statistically significant effect of the test diet containing soybean A5547-127 on body weight (42-day final weight: 2132 g and 2144 g for chickens receiving the conventional counterpart and A5547-127, respectively), body weight gain, feed intake, feed intake/body weight gain or percent mortality. After 42 days, 8 birds were randomly selected from each pen (altogether 48 chicks per treatment) and slaughtered. Birds fed diets containing soybean A5547-127 showed no biologically relevant differences in carcass characteristics (carcass weight, abdominal fat pad weight, deboned breast meat weight, abdominal fat pad weight/carcass weight, and deboned breast meat yield/carcass weight) compared with chickens receiving diets containing seeds of the conventional counterpart (A5547).

Thus, the broiler feeding study supported the results of the comparative compositional analysis and confirmed that seeds produced by soybean A5547-127 are as nutritional as seeds of the conventional counterpart.

5.1.6. Post-market monitoring of GM food/feed

The risk assessment concluded that no data have emerged to indicate that soybean A5547-127 is any less safe than its non-GM comparator. In addition, soybean A5547-127 is, from a nutritional point of view, equivalent to conventional soybean. Therefore, and in line with the Guidance document (EFSA, 2006a), the EFSA GMO Panel is of the opinion that post-market monitoring of the GM food/feed is not necessary.

5.2. Conclusion

The PAT protein is quickly degraded in simulated gastric and intestinal fluids without leaving stable peptide fragments. Bioinformatics-supported studies demonstrated that the PAT protein shows no homology to known toxic and allergenic proteins. The PAT protein induced no toxicity when administered orally to mice in a repeated toxicity study.

Based on the results of a comparative analysis of data, the EFSA GMO Panel concludes that from the compositional point of view, the differences observed between the soybean A5547-127 and the conventional counterpart were not consistent across sites and years and fell within the range of natural variation of soybean. From the phenotypic and agronomic points of view, the EFSA GMO Panel concludes that soybean A5547-127 is phenotypically and agronomically not different from its conventional counterpart A5547, with the exception of the newly introduced trait. Therefore, an

[17] Technical Dossier/ Section 7.10

animal feeding study with the whole soybean was not found necessary. Whole-product testing with sera from soybean-allergic patients showed that the overall allergenicity of soybean A5547-127 is not different from that of commercial soybeans. A feeding study on broiler chickens showed that soybean A5547-127 is as nutritional as soybean A5547. Therefore, the EFSA GMO Panel is of the opinion that soybean A5547-127 is as safe as its conventional counterpart with respect to potential effects on human and animal health and the environment in the context of its intended uses.

6. Environmental risk assessment and monitoring plan

6.1. Environmental risk assessment

The scope of application EFSA-GMO-NL-2008-52 is for food and feed uses, import and processing and does not include cultivation. Considering the intended uses of soybean A5547-127, the environmental risk assessment is concerned with the exposure through manure and faeces from animals fed seed produced by soybean A5547-127 and with the accidental release into the environment of viable seeds of soybean A5547-127 (e.g.; during transportation and processing).

As the scope of the present application excludes cultivation, environmental concerns related to the use of glufosinate-ammonium containing herbicides on soybean A5547-127 apply only to imported and processed soybean products that may have been treated with those herbicides in countries of origin. The EFSA GMO Panel is aware that the risk assessment of active substances falls within the scope of Directive 91/414/EEC concerning the placing of plant protection products on the market.

6.1.1. Unintended effects on plant fitness due to the genetic modification[18]

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

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).

Applicant's field trials have been conducted at several locations in USA during the years 1996, 1997 and 2006 and 2007. These field trials data did not show changes in plant characteristics that indicate altered fitness and invasiveness of GM soybean A5547-127 compared to its conventional counterpart, except in the presence of glufosinate-ammonium containing 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 existing GM soybeans and any change in survival capacity, including overwintering (Dorokhov *et al*., 2004, Owen, 2005, Bagavathiannan and Van Acker, 2008, Lee *et al*., 2009).

Furthermore, there is no evidence that the glufosinate-ammonium containing herbicides tolerance trait introduced by the genetic modification results in increased invasiveness of any crop species, except when glufosinate-ammonium containing herbicides are applied. Thus, the accidental release of GM soybean A5547-127 seeds would not result in the establishment of plants exhibiting dissemination capabilities different from existing conventional soybean varieties and would not create additional agronomic or environmental impacts. The GM soybean plants will only be fitter in the presence of

[18] Technical Dossier / section D9.1

glufosinate-ammonium containing herbicides, which are not currently used in most areas where the GM soybean might be spilled.

Survival of soybean plants outside cultivation areas is mainly limited by a combination of low competitiveness, absence of a dormancy phase, and susceptibility to plant pathogens and cold climate conditions. Since these general characteristics are unchanged in soybean A5547-127, it can be considered that soybean A5547-127 has no altered survival, multiplication or dissemination characteristics, except when glufosinate-ammonium containing herbicides are applied. Therefore, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects of the soybean A5547-127 in Europe will not be different to that of conventional soybean varieties.

6.1.2. Potential for gene transfer[19]

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 micro-organisms in the digestive tract of humans, domesticated animals, and other animals feeding on soybean A5547-127 is expected (see section 4 of the scientific opinion).

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 micro-organisms) is not expected to occur at detectable frequencies under natural conditions (see EFSA, 2009 for more details).

A successful horizontal transfer would require stable insertion of the transgene sequences into a bacterial genome enabling it to multiply at a higher rate than non-transformed cells. The only known mechanism that facilitates horizontal transfer of non-mobile, chromosomal DNA fragments into bacterial genomes is homologous recombination (HR). HR depends on the presence of stretches of similar DNA sequences between the recombining DNA molecules. In addition to substitutive recombination events, HR can also facilitate the insertion of non-homologous DNA sequences into bacterial genomes (additive recombination) if the flanking regions share sequence similarity.

The inserted DNA including the *pat* gene, ColE1 origin of replication and *bla* fragments originates from bacteria and therefore contains sufficient sequence similarity for homologous recombination to take place in related bacterial species. However, such a hypothesised horizontal gene transfer event is not likely to be maintained in bacterial populations due to constraints to efficient expression and a limited advantage for gene transfer recipients in the case of *pat* expression. With regard to the hypothetical case of expression of the *bla* fragments after their possible transfer, they will not encode a functional beta-lactamase protein because the sequences of the *bla* gene present in soybean A5547-127 have been split into two fragments and lack the first 28 bp. In addition to homology-based recombination processes, illegitimate recombination that does not require the presence of DNA similarity between the recombining DNA molecules is theoretically possible. However, the transformation rates for illegitimate recombination were considered to be 10^{10}-fold lower than for homologous recombination (Hülter, 2008, EFSA, 2009). Illegitimate recombination events have not

[19] Technical Dossier / section D9.2

been detected in studies that have exposed bacteria to high concentrations of GM-plant DNA (EFSA, 2009). For these reasons, illegitimate recombination is not further considered here.
The exposure of bacterial communities to the recombinant genes in soybean A5547-127 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.

In the context of its intended uses as food and feed, there is no direct exposure of micro-organisms to the herbicidal compound glufosinate. The selective advantage of glufosinate-ammonium containing herbicides resistance in bacteria is therefore predicted to be limited. The hypothetical rare acquisition of the *pat* from recombinant DNA plants is therefore not considered to confer an advantage to micro-organisms that would allow them to enhance their viability or to alter their habitat range.

The EFSA GMO Panel concludes that the recombinant DNA in soybean A5547-127 does not represent an environmental risk in relation to its potential for horizontal transfer to bacteria and other micro-organisms in the context of its intended uses.

(b) Plant to plant gene transfer

Considering the intended uses of soybean A5547-127 and physical characteristics of soybean seeds, a possible pathway of gene dispersal is from seed spillage and pollen of occasional feral GM soybean plants originating from accidental seed spillage mainly during transportation and/or processing.

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

Soybean (*Glycine max*) is an annual almost completely self-pollinating crop in the field, which has a percentage of cross-pollination usually lower than 1% (Weber and Hanson, 1961, Caviness, 1966, Ray *et al*., 2003, Lu, 2005, Yoshimura *et al*., 2006, Abud *et al*., 2007). Soybean pollen dispersal is limited because the anthers mature in the bud and directly pollinate the stigma of the same flower (OECD, 2000). However, cross-pollination rates as high as 6.3% have been reported for closely spaced plants (Ray *et al*., 2003), suggesting the potential of some within-crop gene flow. These results indicate that natural cross-pollination rates can fluctuate significantly among different soybean varieties under particular environmental conditions, such as favourable climate for pollination and abundance of pollinators (Gumisiriza and Rubaihayo, 1978, Ahrent and Caviness, 1994, Ray *et al*., 2003, Lu, 2005).

Plant to plant gene transfer could therefore occur under the following scenario: imports of soybean A5547-127 seeds (while most soybean A5547-127 seeds will be processed in countries of production), processing outside of importing ports, transportation in regions of soybean production in Europe, spillage of GM seeds mainly during transportation, germination and development of spilled seeds within soybean fields or in very close vicinity of cultivated soybean fields, overlap of flowering periods and environmental conditions favouring cross-pollination. The likelihood of all these conditions occurring and thereby resulting in cross-pollination between GM soybean plants and cultivated soybean is therefore extremely low. Apart from seed production areas, GM plants and plants derived from out-crossing with this GM soybean will not persist overtime. Dispersal of soybean seeds

by animals is not expected due to the characteristics of the seed, but accidental release into the environment of seeds may occur (e.g.; during transportation and processing for food, feed and industrial uses). However, cultivated soybean seeds rarely display any dormancy characteristics and only under certain environmental conditions do they grow as volunteers in the year following cultivation (OECD, 2000). Even in soybean fields, seeds usually do not survive during the winter due to predation, rotting, germination resulting in death, or due to management practices prior to planting the subsequent crop (Owen, 2005).

The EFSA GMO Panel takes into account that this application does not include cultivation of the soybean within the EU so that the likelihood of cross-pollination between cultivated soybean and occasional soybean plants resulting from seed spillage is considered extremely low. However, in countries cultivating this GM soybean and producing seed for export, there is a potential for admixture in seed production and thus the introduction of GM seeds through this route. Hence, it is important that appropriate management systems are in place to restrict seeds of soybean A5547-127 entering cultivation as this would require specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

In conclusion, since soybean A5547-127 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 A5547-127 in Europe will not differ from that of conventional soybean varieties.

6.1.3. Interactions of the GM plant with target organisms

Due to the type of trait (glufosinate-ammonium containing herbicides tolerance with no target organisms) and the intended uses of soybean A5547-127, which exclude cultivation, this was not considered as an issue by the EFSA GMO Panel.

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

Due to the intended uses of soybean A5547-127, which exclude cultivation and due to the low level of exposure to the environment, potential interactions of the GM plant with non-target organisms were not considered an issue by the EFSA GMO Panel.

6.1.5. Interactions with the abiotic environment and biogeochemical cycles

Due to the intended uses of soybean A5547-127, which exclude cultivation and due to the low level of exposure to the environment, potential interaction of the GM plant with the abiotic environment and biogeochemical cycles were not considered an issue by the EFSA GMO Panel.

6.1.6. Monitoring[20]

The objectives of a monitoring plan according to Annex VII of Directive 2001/18/EC are 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 to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment which were not anticipated in the environmental risk assessment.

Monitoring is related to risk management, and thus a final adoption of the monitoring plan falls outside the mandate of EFSA. However, the EFSA GMO Panel gives its opinion on the scientific

[20] Technical Dossier / section D11

content of the monitoring plan provided by the applicant (EFSA, 2006b). The potential exposure to the environment of soybean A5547-127 would be through manure and faeces from animals fed with GM soybean or through accidental release into the environment of GM soybean seeds (e.g.; during transportation and processing). The EFSA GMO Panel is aware that, due to physical characteristics of soybean seed and methods of transportation, accidental spillage cannot be excluded. Therefore, the EFSA GMO Panel recommends that appropriate management systems are introduced to actively monitor the occurrence of feral soybean plants in areas where soybean spillage and plant establishment are likely to occur as proposed in the EFSA Guidance Document (EFSA, 2006a) and the scientific opinion of the EFSA GMO Panel on post-market environmental monitoring (EFSA, 2006b).

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

The general surveillance plan proposed by the applicant includes: (1) the description of an approach involving operators (federations involved in soybean import and processing) reporting to the applicant via a centralised system any observed adverse effect(s) of GMOs on human health and the environment, (2) a coordinating system established by EuropaBio for the collection of information recorded by the various operators (Lecoq *et al*., 2007, Windels *et al*., 2008), (3) the use of networks of existing surveillance systems. The applicant proposes to submit a general surveillance report on an annual basis and a final report at the end of the consent.

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 A5547-127 since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

6.2. Conclusion

The scope of application EFSA-GMO-NL-2008-52 is for food and feed uses, import and processing of soybean A5547-127 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 A5547-127 and with the accidental release into the environment of viable seeds of soybean A5547-127 (e.g.; during transportation and processing).

In case of accidental release into the environment of viable seeds of soybean A5547-127 (e.g. during transportation and processing), there are no indications of an increased likelihood of establishment and spread of feral soybean A5547-127 plants, except in the presence of glufosinate-ammonium containing herbicides. In addition, the low levels of environmental exposure of these GM soybean plants and the newly expressed protein through other routes indicate that the risk to non-target organisms is extremely low. The risk caused by a possible transfer of the recombinant gene from soybean A5547-127 to environmental micro-organisms is regarded to be negligible due to the lack of a advantage that would be conferred 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 A5547-127.

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

The EFSA GMO Panel also recommends that appropriate management systems should be in place to restrict seeds of soybean A5547-127 entering cultivation as this would require specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

CONCLUSIONS [AND/OR] RECOMMENDATIONS

The EFSA GMO Panel was requested to carry out an evaluation of a scientific risk assessment of the soybean A5547-127 for food and feed uses, import and processing in accordance with Regulation (EC) No 1829/2003.

The EFSA GMO Panel is of the opinion that the molecular characterisation data provided for soybean A5547-127 are sufficient to conclude on this part of the risk assessment evaluation. The results of the bioinformatic analyses of the inserted DNA and the flanking regions do not raise safety concerns. The levels of PAT protein in soybean A5547-127 have been sufficiently analysed in various tissues and the stability of the genetic modification has been demonstrated. The EFSA GMO Panel considers that the molecular characterisation does not indicate a safety concern.

Based on the results of a comparative analysis of data, the EFSA GMO Panel concludes that from the compositional point of view, the differences observed at some field trial sites between the soybean A5547-127 and the conventional counterpart were not consistent across sites and years and fell within the range of natural variation of soybean. From the phenotypic and agronomic points of view, the EFSA GMO Panel concludes that soybean A5547-127 is phenotypically and agronomically not different from its conventional counterpart A5547, with the exception of the newly introduced trait. The PAT protein expressed in soybean A5547-127 is degraded in simulated digestive and intestinal fluids, and bioinformatics-supported studies demonstrated that the PAT protein show no homology to known toxic and allergenic proteins. No toxicity of the PAT protein was observed in a repeated toxicity study in mice where the protein was administered orally at a high dose.

Whole-product testing of soybean extracts to sera from soy-allergic patients demonstrated unchanged overall allergenicity of the whole plant. A 42-day feeding study on broiler chickens showed that soybean A5547-127 is as nutritional as its conventional counterpart.

Considering the intended uses of soybean A5547-127, which exclude cultivation, there is no requirement for scientific assessment on possible environmental effects associated with the cultivation of this GM soybean. In case of accidental release into the environment of viable seeds of soybean A5547-127 (e.g.; during transportation and processing), there are no indications of an increased likelihood of establishment and spread of feral soybean plants, except in the presence of glufosinate-ammonium containing herbicides. In addition, the low levels of environmental exposure of these GM soybean plants and the newly expressed protein through other routes indicate that the risk to non-target organisms is extremely low. The risk caused by a possible transfer of the recombinant gene from soybean A5547-127 to environmental micro-organisms is regarded to be negligible due to the lack of any selective advantage that would be conferred 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 A5547-127. The EFSA GMO Panel is aware that, due to physical characteristics of soybean seed and methods of transportation, accidental spillage cannot be excluded. Therefore, the EFSA GMO Panel recommends that, within general surveillance, appropriate management systems are introduced to actively monitor the occurrence of feral soybean plants in areas where soybean spillage and plant establishment are likely to occur.

The EFSA GMO Panel recommends that appropriate management systems should be in place to restrict seeds of soybean A5547-127 entering cultivation as the latter requires specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

In conclusion, the EFSA GMO Panel considers that the information available for soybean A5547-127 addresses the scientific comments raised by the Member States and that the soybean A5547-127, as described in this application, is as safe as its conventional counterpart with respect to potential effects on human and animal health and the environment in the context of its intended uses.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of The Netherlands, received 3 April 2008, concerning a request for placing on the market of Soybean A5547-127 submitted by Bayer under Regulation (EC) No 1829/2003.

2. Acknowledgement letter, dated 15 April 2008, from EFSA to the Competent Authority of The Netherlands (Ref.KL/shv(2008) 2930176).

3. Letter from EFSA to applicant, dated 30 May 2008, requesting additional information under completeness check (Ref.PB/CP/shv(2008) 3058580).

4. Letter from applicant to EFSA, received 5 June 2008, providing additional information under completeness check.

5. Letter from EFSA to applicant, dated 13 June 2008, requesting additional information under completeness check (Ref.PB/CP/shv(2008) 3097126).

6. Letter from applicant to EFSA, received 26 June 2008, providing additional information under completeness check.

7. Letter from EFSA to applicant, dated 18 July 2008, delivering the 'Statement of Validity' for application EFSA-GMO-NL-2008-52, Soybean A5547-127 submitted by Bayer under Regulation (EC) No 1829/2003 (Ref.PB/CP/md (2008) 3181534).

8. Letter from EFSA to applicant, dated 5 December 2008, requesting additional information and stopping the clock (Ref.PB/CP/shv(2008) 3509427).

9. Letter from applicant to EFSA, received 16 April 2009, providing additional information.

10. Letter from EFSA to applicant, dated 29 June 2009, requesting additional information and maintaining the clock stopped (Ref.PB/CP/ls(2009) 4073485).

11. Letter from applicant to EFSA, received 9 December 2010, providing additional information.

12. Letter from EFSA to applicant, dated 7 March 2011, restarting the clock (Ref.PB/KL/CP/lg (2011) 5614099).

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Capt A. 2007c. PAT protein from pat gene, Overall amino acid sequence homology search with known toxins and allergens. Bayer CropScience Internal Report. 163 pages. M- 266573-02-1

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