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Opinion of the Scientific Panel on Genetically Modified Organisms on an application (Reference EFSA-GMO-NL-2005-18) for the placing on the market of the glufosinate tolerant soybean A2704-12, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience[1]

(Question No EFSA-Q-2005-174)

Opinion adopted on 3 July 2007

SUMMARY

This document provides an opinion of the Scientific Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority (EFSA) on genetically modified soybean A2704-12 (Unique Identifier ACS-GMØØ5-3) developed to provide tolerance to glufosinate herbicides.

In delivering its opinion the GMO Panel considered the application EFSA-GMO-NL-2005-18, additional information provided by the applicant (Bayer CropScience) and the scientific comments submitted by the Member States. The scope of application EFSA-GMO-NL-2005-18 is for food and feed uses, import and processing of soybean A2704-12 and its derived products for the same uses as any non-GM soybean (food, feed including meal, cake and oil), excluding cultivation.

The GMO Panel assessed soybean A2704-12 with reference to its intended uses. The assessment followed 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. The scientific assessment included molecular characterization of the inserted DNA and expression of the PAT protein. A comparative analysis of agronomic traits and composition was undertaken. The potential toxicity and allergenicity of the new protein was evaluated together with the potential allergenicity of extracts of whole food/feed. Environmental assessment was undertaken, including a monitoring plan.

Soybean A2704-12 is derived from the soybean variety A2704 which was transformed using particle bombardment. Soybean A2704-12 expresses the *pat* gene leading to the production of the enzyme phosphinothricin acetyl-transferase (PAT) that acetylates L-glufosinate-ammonium. The PAT enzyme confers tolerance to glufosinate herbicides.

The molecular characterisation data established that soybean A2704-12 contains a single insert. The insert contains all of the sequences of plasmid vector pB2/35SAcK, including the vector backbone consisting of pUC19 sequences. The insert also contains two separate (3' and 5') fragments of the *bla* gene. These fragments do not constitute a functional gene in soybean A2704-12 and the *bla* gene was not expressed.

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Bioinformatics analysis was carried out on open reading frames (ORFs) spanning the insert, the insert/genomic junctions and the 5' chloroplastic/genomic junction regions. This analysis showed that it is unlikely that the eight putative ORFs are expressed in soybean A2704-12. Even in the unlikely event that expression did occur, any resulting polypeptides would show no significant sequence homology with known toxins or allergens. The GMO Panel is of the opinion that the molecular characterisation of the DNA insert and flanking regions of soybean A2704-12 does not raise safety concerns, and sufficient evidence for the stability of the insert structure and of the newly introduced trait was provided.

The GMO Panel assessed the results of the compositional analysis of A2704-12 soybean and its comparator in the light of the field trial design, measured biological variation and the level of the studied compounds in conventional soybean varieties, and concludes that A2704-12 soybean is compositionally equivalent to the non-GM counterpart except for the introduced trait.

No toxicity of the PAT protein was observed in a single dose acute toxicity study in mice using intravenous injection. Furthermore, the PAT protein is rapidly degraded under simulated gastric and intestinal conditions. The PAT protein shows no homology with known toxic proteins and/or allergens. The PAT protein has been extensively assessed in previous opinions of the EFSA GMO Panel and no concerns were raised concerning the safety of the PAT protein. Furthermore *in vitro* allergenicity testing of extracts of A2704 12 soybeans showed no changes in allergenicity.

The application EFSA-GMO-NL-2005-18 is for food and feed uses, import and processing. There is therefore no requirement for scientific information on possible environmental effects associated with the cultivation of soybean A2704-12. Considering the scope of the application, the GMO Panel is of the opinion that the likelihood of the spread and establishment of soybean A2704-12 is very low and that unintended environmental effects due to this soybean will be no different from that of conventional soybean varieties. The scope of the monitoring plan provided by the applicant and the reporting intervals are in line with the intended uses of soybean A2704-12 since cultivation is excluded.

In conclusion, taking into account all of the data assessed, the GMO Panel considers that the information available for soybean A2704-12 addresses the scientific comments raised by the Member States and that the soybean A2704-12 is as safe as its non genetically modified counterpart with respect to potential effects on human and animal health or the environment. Therefore the GMO Panel concludes that soybean A2704-12 is unlikely to have any adverse effect on human and animal health or on the environment in the context of its intended uses.

Key words: GMO, maize, glufosinate tolerance, PAT protein, *pat* gene, ACS-GMØØ5-3, human and animal health, environment, import, food, feed, Regulation (EC) No 1829/2003.

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BACKGROUND

On 13 July 2005 EFSA received from the Dutch Competent Authority an application (Reference EFSA-GMO-NL-2005-18), for authorisation of soybean A2704-12 (Unique Identifier ACS-GMØØ5-3), submitted by Bayer CropScience within the framework of Regulation (EC) No 1829/2003 on genetically modified food and feed (EC, 2003).

After receiving the application EFSA-GMO-NL-2005-18 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 dossier available to the public 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 1 February 2006, EFSA received additional information (requested 20 December 2005) and declared the application as formally valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003 on 10 February 2006.

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. The Member State bodies had three months after the date of receipt of the valid application (until 10 May 2006) within which to make their opinion known.

On 14 February 2006, 6 July 2006, 24 October 2006, 23 January 2007 and 23 May 2007, the GMO Panel asked for additional data on soybean A2704-12. The applicant provided the requested information on 2 and 8 August 2006, 17 November 2006 and 30 May 2007. After receipt and assessment of the full data, the GMO Panel finalized its risk assessment of soybean A2704-12.

The GMO Panel carried out a scientific assessment of the genetically modified soybean A2704-12 for food and feed uses and import and processing, in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003, taking into consideration the scientific comments of the Member States and the additional information provided by the applicant.

In giving its opinion on soybean A2704-12 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 receipt 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, the EFSA opinion shall include a report describing the assessment of the food and feed and stating the reasons for its opinion and the information on which its opinion is based. This document is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation and thus will be part of the overall opinion in accordance with Articles 6(5) and 18(5).

TERMS OF REFERENCE

The GMO Panel was requested, in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003, to carry out a scientific assessment of the genetically modified soybean A2704-12 for food and feed uses, import and processing. Soybean A2704-12 will be imported in the European Union as commodity soybean and derived products, including meal, cake and oil for use as food and feed uses.

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 GMO Panel was not requested to give an opinion on information required under Annex II to the Cartagena Protocol. The 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 A2704-12 (Unique Identifier ACS-GMØØ5-3) was assessed 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).

2. Molecular characterisation

2.1. Issues raised by the Member States

Comments were provided regarding the need of further characterisation of the 5' flanking region, including extended sequencing and bioinformatic analysis of the insert/genomic DNA junctions.

2.2. Evaluation of relevant scientific data

2.2.1. Transformation process and vector constructs

Tissue from embryo shoot apices derived from surface-sterilized soybean seeds was transformed by *Pvu*I digested vector plasmid pB2/35SAcK using particle bombardment. The vector pB2/35SAcK is derived from pUC19, and contains the complete pUC19 vector with, among others, the ColE1 origin of replication and the *bla* gene conferring resistance to ampicillin in bacteria. In addition, the plasmid vector contains a right

border fragment from *Agrobacterium tumefaciens* Ti plasmid TiAch5 and a *pat* expression cassette. *Pvu*I digestion of the vector construct pB2/35SAcK was performed before transformation to disrupt the coding sequence of the *bla* gene. The *Pvu*I digestion generated two fragments, one containing the PAT cassette and the 3' part of the *bla* gene and the second containing the 5' part of the *bla* gene and the right border fragment. The digested pB2/35SAcK was used for the transformation.

The *pat* expression cassette consists of the following elements: the P35S promoter from cauliflower mosaic virus, the synthetic *pat* gene derived from the *pat* gene of *Streptomyces viridochromogenes* resulting in tolerance to glufosinate ammonium-containing herbicides, and the T35S terminator from cauliflower mosaic virus. The *pat* gene in vector pB2/35SAcK consists of a synthetic version of the *S. viridochromogenes bar* gene optimised for expression in plants. The *Sal*I site preceding the ATG start codon was removed. This modification has no effect on the resulting amino acid sequence of the PAT protein.

2.2.2. Transgenic constructs in the genetically modified plant

Southern analysis of genomic DNA digested with combinations of six different restriction enzymes was performed using four probes that cover the inserted *pat* and *bla* sequences. This analysis demonstrated that the insert consists of two copies of the *pat* expression cassette in a single locus. Between the two *pat* cassettes one copy of the *Pvu*I digested 3' part of the *bla* sequence and one copy of the 5' part of *bla* sequence is inserted. The 5' *bla* sequence is integrated in a reverse orientation with respect to the 3' *bla* sequence. Therefore the arrangement of the two parts of the *bla* sequence in soybean A2704-12 does not constitute a functional *bla* gene.

The nucleotide sequence of the insert in soybean A2704-12 has been determined in its entirety and consists of 6780 bp. The sequence is identical to the corresponding DNA sequences of the transforming plasmid vector pB2/35SAcK.

The 3' and 5' flanking sequences of the insert were amplified by PCR and sequenced, confirming the presence of soybean DNA. BLAST analysis of the 3' sequence did not reveal significant similarity with any known soybean gene. Analysis of 2466 bp 5' sequence demonstrated homologies to part of the soybean chloroplast 16S-23S spacer DNA. The integration of plastid DNA within the nuclear plant genome is not uncommon and the GMO Panel considered that this *per se* is unlikely to be a safety concern.

Sequencing was extended further into the flanking 5' genomic region (1478 bp) which was shown to correspond to soybean genomic DNA. A similarity search carried out on this region showed matches to an *Arabidopsis thaliana* expressed sequence tag (EST) corresponding to a gene of unknown function. The sequence is located entirely within the 5' flanking region and is not disrupted by the transgene insertion.

A fragment of 2082 bp of soybean genomic DNA was deleted at the insertion site in soybean A2704-12. Bioinformatic analysis of the deleted genomic sequence demonstrated partial deletion of a putative gene. Homology searches suggest that this putative gene encodes a transcription factor, occurring in *Arabidopsis thaliana* as a small multigene family. The observed deletion did not result in any unexpected phenotypic, agronomic or compositional changes during the breeding process (see sections 3.2.2, 3.2.3).

2.2.3. Information on the expression of the insert

2.2.3.1. Expression of the introduced genes

Northern analysis was performed to determine whether the *bla* sequences were expressed in soybean A2704-12. No transcription of the *bla* sequences in soybean A2704-12 was demonstrated. Analysis of PAT protein was carried out by ELISA using plants grown under greenhouse and field conditions. Expression could be demonstrated in roots, stems, seeds and leaves of soybean A2704-12. Under greenhouse conditions the mean PAT concentrations in unsprayed plants ranged from 0.30 to 3.69 µg/g fresh weight (FW) in roots, 4.86 to 10.0 µg/g FW in stems and 11.7 to 17.6 µg/g FW in leaves. Average levels of PAT protein in soybean seeds under field conditions over 12 different field locations in the United States and Canada during the years 1996-1999 were 478 to 2382 ng/g FW of seed. The PAT content of the seeds measured in 1996 and 1999, averaged over locations, was not significantly influenced by herbicide application.

2.2.3.2. Putative cryptic open reading frames (ORF)

Bioinformatic analysis (BLAST searches) of the A2704-12 insert sequence was performed to indicate the presence of putative open reading frames 1) within the insert, 2) spanning the junctions between the 3' and 5' flanking DNA/insert junctions and 3) spanning the 5' flanking chloroplast/genomic DNA junction region. An ORF was defined by the applicant as any region between a start and stop codon with a minimum size of 3 amino acids. Two ORFs (ORF-1 and ORF-2) were found at the 5' flanking region of the inserted DNA, and five ORFs (ORF-3 to ORF-7) were created within the insert in A2704-12 at the junctions between the *Pvu*I fragments. In addition, one ORF (ORF-8) was found at the 5' flanking chloroplast/genomic DNA junction region. No ORFs were detected at the 3' flanking region of the insert. The presence of putative ORFs raises the possibility that new putative fusion proteins could be produced. Further analysis revealed it to be unlikely that the putative ORFs are transcriptionally and/or translationally active. Bioinformatic analysis of these eight ORF sequences showed no sequence homology with known toxins or allergens. The presence of these putative ORFs is unlikely to raise any safety concerns.

2.2.4. Inheritance and stability of inserted DNA

The inheritance of the introduced trait in soybean A2704-12 follows a Mendelian pattern characteristic of a dominant single gene. Stability and possible changes of the organisation of the inserted DNA was studied over 3 generations in soybean A2704-12. Plant DNA was digested with *Hind*III or *Nco*I restriction enzymes, since both enzymes have only one restriction site in the insert. Southern analysis using the *pat* gene as a probe revealed the presence of the expected part of the insert containing either the 3' (*Nco*I digest) or 5' flanking DNA (*Hind*III digest) after 3 generations. This indicated that the inserted DNA and the DNA flanking the insert are stable in soybean A2704-12 over at least 3 generations. To further determine the stability of the insert plants were grown under greenhouse conditions and in the field for 8 to 9 generations at a minimum of 3 geographic locations. The event A2704-12 was also introduced in 6 different genetic backgrounds of soybean using conventional breeding. Plants were grown in at least 3 different geographic locations for 12 generations. Southern analysis with *EcoR*V digested genomic DNA revealed the expected hybridisation pattern using the *pat* gene as a probe.

These results indicate genetic and molecular stability of the insert present in the soybean A2704-12 in different genetic backgrounds, over several generations and under different environmental conditions.

2.3. Conclusion

The molecular characterisation data established that soybean A2704-12 contains a single insert. The insert contains all the sequences of plasmid vector pB2/35SAcK, including the vector backbone consisting of pUC19 sequences. The insert also contains two separate (3' and 5') fragments of the *bla* gene. These fragments do not constitute a functional gene in soybean A2704-12 and the *bla* gene was not expressed. Bioinformatics analysis of ORFs spanning the insert, the insert/genomic junctions and the 5' chloroplastic/genomic junction regions in the GM soybean showed that it is unlikely that the eight putative ORFs are expressed in soybean A2704-12. Even in the unlikely event that expression occurs any resulting polypeptides would show no significant sequence homology with known toxins or allergens.

The GMO Panel is of the opinion that the molecular characterisation of the DNA insert and flanking regions of soybean A2704-12 does not raise safety concerns, and sufficient evidence for the stability of the insert structure and of the newly introduced trait was provided.

3. Comparative analysis

3.1. Issues raised by Member States

Questions were raised regarding a) the variation in vitamin E content in both non-treated and glufosinate-treated GM soybean material from the different trial sites b) the relatively higher content of some isoflavones in GM soybean compared with the literature data and c) the statistical analysis of the compositional data.

3.2. Evaluation of relevant scientific data

Having considered the information provided in the application and the Member States comments, the GMO Panel requested from the applicant further data with respect to the statistical analysis (ANOVA) of the compositional data and an explanation of the observed variation in vitamin E content between materials from various locations. The applicant provided the requested statistical analysis and additional data explaining the variation in vitamin E content.

3.2.1. Choice of comparator and production of material for the compositional assessment

For compositional studies, A2704-12 soybean was compared to the non-transgenic parental variety A2704, which is a commercial soybean variety extensively grown in the US because of its good agronomic performance. The field trials were carried out during the year 1999 in Illinois, Nebraska, Wisconsin and Ontario and during the year 2000 in Iowa, Indiana, Wisconsin, Minnesota and Ontario. Three replicates were used for each of the three treatments (non-GM A2704 soybean, GM A2704-12 soybean not sprayed, GM A2704-12 sprayed with glufosinate ammonium) at each trial site.

3.2.2. Compositional analysis

Soybean seeds were collected during field trials for compositional analysis. The compositional analysis of A2704-12 soybean and its non-transgenic counterpart A2704 was carried out with respect to proximates, fibre compounds, micro-nutrients (minerals, vitamins), amino acids, fatty acids, anti-nutrients (i.e. phytic acid, trypsin inhibitors, lectins, stachyose and raffinose), and other secondary metabolites (isoflavones). The set of compounds analysed was in agreement with the key nutrients, anti-nutrients, and toxicants recommended by OECD (OECD, 2001). An additional analysis (ANOVA) of compositional parameters has been provided by the applicant in response to a request by the GMO Panel. ANOVA analysis on the collected data comparing the three treatments showed statistically significant increases/reductions in the level of several of all the compounds analyzed. However, these differences were present only in material from few locations. Furthermore, when data from the two locations where soybean was planted in both years were analysed, the endpoints for only two metabolites showed statistically significant differences: arginine and linoleic acid C18:2. However, a direct comparison of the observed differences with the ranges of natural variation showed that the values were within the ranges published in the literature (OECD, 2001).

The statistical analysis (ANOVA) showed that there were no significant differences in E vitamin content between the non-GM, non sprayed GM and GM soybean sprayed with glufosinate ammonium. However, between locations differences in vitamin E content were observed, but these differences are likely attributable to climatic conditions (Almonor *et al*., 1998).

Variability in isoflavone content of both A2704-12 soybean and A2704 soybean control was observed. This is in line with the scientific literature, which reports that isoflavone contents of soybean can fluctuate depending on various conditions, including location and genotype *(e.g.* Riedl *et al.*, 2007). Although there were some statistically significant differences between the GM and non-GM soybean within some locations, this was not observed consistently in each location. In addition the values of isoflavone content were within the ranges reported by the OECD (OECD, 2001).

In addition to the analysis of soybean seeds the applicant analysed hay, forage, hulls, soy meal, toasted meal, protein isolate, refined oil, and crude lecithin. Compositional similarities between soybean A2704-12 and its corresponding control were confirmed in the analysis of processed food and feed commodities.

The GMO Panel considered the observed compositional differences between A2704-12 soybean and its comparator in the light of the field trial design, measured biological variation and the level of the studied compounds in conventional soybean varieties, and concludes that A2704-12 soybean is compositionally equivalent to the non-GM counterpart except for the introduced trait.

3.2.3. Agronomic traits and GM phenotype

The applicant provided information on agronomic performance and phenotypic characteristics of genetically modified and non-genetically modified soybean derived from field trials at 3 locations in the US in 1996 and in Southern Ontario (Canada) in 1998. The characteristics that were analyzed in these studies were plant height, yield, and plant, seed, and flower morphology. In the US field trials plant maturity was also measured. The GMO Panel assessed the data provided and considers GM A2704-12 soybean to be agronomically equivalent to the currently grown non-GM A2704 soybean, with the exception of the newly introduced trait.

3.3. Conclusion

Analyses carried out on materials from A2704-12 soybean and its closely genetically related comparator indicated that these soybeans are compositionally and agronomically equivalent except for the introduced transgenic trait. The comparative analysis of A2704-12 soybean provided no indication of unintended effects resulting from the genetic modification.

4. Food/Feed safety assessment

4.1. Issues raised by Member States

Member States raised issues regarding a) the use of a PAT protein produced in bacteria in the safety studies instead of the plant expressed PAT protein, b) the lack of subchronic toxicity studies using the whole GM food/feed, c) the short duration of the nutritional study in broiler chickens and d) the allergenicity testing of the newly expressed PAT protein, including bioinformatics analysis.

4.2. Evaluation of relevant scientific data

4.2.1. Product description and intended use

The scope of application EFSA-GMO-NL-2005-18 is for food and feed uses, import and processing of A2704-12 soybean and its derived products, including oil, meal and cake. Soybeans can be used directly in various animal feeds.

The modification of A2704-12 soybean is intended to improve agronomic performance only and is not intended to influence the nutritional aspects, production processes and overall use of soybean as a crop.

4.2.2. Stability during processing

The effect of temperature on recombinant PAT protein produced by *E. coli* was assessed by SDS-PAGE electrophoresis following incubation for up to 60 minutes at 60, 75 and 90°C. No degradation of the PAT protein was observed under these temperature conditions. However, PAT protein was not detectable by ELISA in processed soybean products such as defatted and toasted meals, crude lecithin, refined oil, or refined bleached and deodorized oil. Given the toxicological profile and allergenic properties of PAT protein the Panel is of the opinion that no further stability analyses are required.

4.2.3. Toxicology

4.2.3.1. PAT protein used for safety assessment

Due to the low expression level of the PAT protein in soybean A2704-12 and the very difficult task of isolating a sufficient quantity of purified protein from this soybean, protein safety studies were conducted

with a PAT protein encoded by the *pat* gene (PAT/*pat* protein) and expressed in *E. coli*. The equivalence of the PAT protein produced by *E. coli* and plant was proven by Edman degradation, western blot analysis, protein mobility in SDS-PAGE, mass spectrometry, and enzymatic activity. All of these methods confirmed the equivalence of the bacterial and the plant PAT protein. Based on the identified similarity in structure and function between these proteins, the GMO Panel accepts the use of PAT/*pat* test material derived from *E. coli* for the safety testing of the PAT protein present in soybean A2704-12.

On a request from the Panel the applicant confirmed that the PAT protein used in the digestibility and toxicity studies was consistently the same protein.

4.2.3.2. Toxicological assessment of expressed novel protein

(a) Acute toxicity testing

The applicant provided a single dose toxicity study on female mice intravenously injected with 1 or 10 mg per kg body weight of the PAT/pat protein. The intravenous injection was chosen because of the expected fast proteolytic degradation of the protein in digestive environments. Animals were observed for 15 days after dosing and macroscopic examination of internal organs was carried out at necropsy. Even at the relatively high dose of 10 mg/kg body weight, no signs of systemic toxicity were observed.

(b) Degradation in simulated digestive fluids

The PAT/pat protein was used in the *in vitro* digestibility test in simulated gastric fluid containing pepsin and incubated. Degradation occurred rapidly, (within 30 seconds at pH 2) as demonstrated by Coomassie blue staining of proteins following SDS –PAGE gel electrophoresis.

Rapid degradation (within seconds) of the 25kDa PAT/pat protein was also demonstrated by Western blots after incubation in simulated intestinal fluid (pH 7.5) containing pancreatin. During degradation residual fragments of 5 to 14 kDa appeared transiently. These fragments disappeared after 5 minutes of incubation.

The *in vitro* digestion experiments demonstrate that the PAT/pat protein is rapidly degraded in simulated gastric and intestinal conditions.

(c) Bioinformatic studies

Searches for amino acid sequence homology of the PAT protein in soybean A2704-12 with sequences from protein databases indicated significant homology only with other acetyltransferases. There was no sequence homology with known toxic proteins.

4.2.3.3. Toxicological assessment of new constituents other than proteins

No new constituent other than the PAT protein is expressed in soybean A2704-12 and no relevant changes in composition were detected by the compositional analysis.

4.2.4. Toxicological assessment of the whole GM food/feed

No oral toxicity studies with the whole GM food/feed were provided.

The GMO Panel after considering all the data available on the molecular characterization, compositional analysis and agronomic performance, came to the conclusion that soybean A2704 12 is equivalent to its non GM counterpart. Therefore, the Panel did not see the need for further animal safety studies with the whole food/feed.

4.2.5. Allergenicity

The strategies used when assessing the potential allergenic risk focus on the characterisation of the source of the recombinant protein, the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons and whether the transformation may have altered the allergenic properties of the modified 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, 2004).

4.2.5.1. Assessment of allergenicity of the newly expressed proteins

Pat gene originates from *Streptomyces viridochromogenes,* a soil microrganism that is not known to be allergenic. The PAT protein was subjected to bioinformatics analysis. The results of sequence homology search for identical sequences of at least 8 contiguous amino acids showed no similarities between known allergens and the PAT protein expressed by A2704-12 soybean. The PAT protein has a high sequence similarity only with other acetyltransferases not known to be allergenic, PAT is not stable in an acidic environment and is rapidly degraded under simulated gastric and intestinal conditions. Based on these results the GMO Panel considers that the newly expressed PAT protein is not likely to be allergenic.

4.2.5.2. Assessment of allergenicity of the whole GM plant

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 (with the exception of the introduced trait) to the conventional comparator was demonstrated on the basis of compositional analysis, no increased allergenicity is anticipated for soybean A2704-12. The applicant has also performed *in vitro* allergenicity study with A2704-12 soybean using sera from patients reactive to soy. These studies showed no change in allergenicity to soybean due to genetic modification (Lehrer, 1997). The GMO Panel concludes that the information presented confirms that the overall allergenicity of the whole plant is not changed.

4.2.6. Nutritional assessment of GM food/feed

The comparative compositional and agronomic analysis of GM 2704-12 soybean and its non-GM comparator showed that GM soybean is compositionally and agronomically equivalent to the non-GM

comparator and provided no indication for unintended effects of the genetic modification. Therefore the GMO Panel concludes that no nutritional studies are needed (EFSA, 2006a).

However, the applicant has provided a 15-day broiler feeding study instead of the generally accepted 42-day broiler feeding study for fattening (ILSI, 2003). Data on body weight gain and feed intake did not indicate differences between treatments groups (GM vs. non GM soybean). Although the applicant argued that growing broilers are sensitive test species in which a 15 fold increase in body weight occurs during the first 18 days of life, the GMO Panel is of the opinion that this study is of limited value for the nutritional assessment, given the short duration of the study and the limited number of endpoints considered.

4.2.7. Post-market monitoring of GM food/feed

The risk assessment concluded that no data have emerged to indicate that 2704-12 soybean is any less safe than its non-GM comparator. In addition, 2704-12 soybean is compositionally equivalent to conventional soybean. Therefore, and in line with the Guidance document (EFSA, 2006a), the GMO Panel is of the opinion that post-market monitoring of the GM food/feed is not necessary.

4.3. Conclusion

No toxicity of the PAT protein was observed in a single dose acute toxicity study in mice using intravenous injection. Furthermore, the PAT protein is rapidly degraded under simulated gastric and intestinal conditions. The PAT protein shows no homology with known toxic proteins and/or allergens. Furthermore, the PAT protein has been extensively assessed in previous opinions of the EFSA GMO Panel and found to be safe (EFSA 2005a,b,c, 2006c, 2007). No concerns were raised concerning the safety of the PAT protein.

The comparative compositional analyses showed that A2704-12 soybean is compositionally equivalent to the non-GM comparator. The composition was also comparable to that of other commercial soybean varieties.

The GMO Panel is of the opinion that 2704-12 soybean is as safe as its non GM counterparts and that the overall allergenicity of the whole plant is not changed. The GMO Panel considers that no additional animal safety or nutritional study is needed.

5. Environmental risk assessment and monitoring plan

5.1. Issues raised by Member States

Comments were provided regarding persistence and invasiveness, gene transfer to symbiotic bacteria and potential effects of glufosinate-containing herbicides on biodiversity.

Further comments were raised with respect to the environmental monitoring plan regarding in particular a more detailed general surveillance plan.

5.2. Evaluation of relevant scientific data

5.2.1. Environmental risk assessment

The scope of this application EFSA-GMO-NL-2005-18 is for food and feed uses, import and processing and excludes cultivation. Therefore, the environmental risk assessment is limited to accidental release into the environment of GM soybean seeds during transportation and processing for food and feed uses.

As this application is not for cultivation, concerns regarding the use of glufosinate-containing herbicides on soybean A2704-12 apply only to imported and processed soybean that may have been treated with these glufosinate-containing herbicides in the countries of origin. The risk assessment of these herbicides is within the scope of Directive 91/414/EEC concerning the placing on the market of plant protection products (EC, 1991).

5.2.1.1. Potential unintended effects on plant fitness due to the genetic modification

Cultivated soybean species *(Glycine max (L.) Merr.)* belong to the subgenus *Soja* of the genus *Glycine*. The species originated from eastern Asia and is a highly domesticated crop (Lu, 2005). The major worldwide soybean producers are the United States, Brazil, Argentina and China. In Europe, soybean is mainly cultivated in Italy, France and Romania (FAOSTAT, 2005). Weedy soybean has not been reported growing naturally outside its centre of origin in other parts of the world such as the Americas and Europe where only the cultivated soybean is commercially grown (Lu, 2005).

Seed and pollen are potential sources of gene dispersal. 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; Lu, 2005).

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 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 grow as a volunteer in the year following cultivation (OECD, 2000). Even in the agroecosystem soybean seeds usually do not survive due to predation, rotting, germination resulting in death during the winter, or due to management practices prior to planting the subsequent crop (Owen, 2005).

In the event of an accidental release and establishment of soybean A2704-12 in the environment, the GM soybean plants will only be fitter when cultivated in the presence of glufosinate-containing herbicides which are not currently used on cultivated soybean or in most areas where the GM soybean might be spilled.

The data presented in the application indicate that, in the field studies carried out in United States and Canada during the years 1996 (2 sites), 1998 (2 sites) and 2002 (1 site), soybean A2704-12 has no altered survival, multiplication or dissemination characteristics compared to its conventional counterparts except in the presence of glufosinate herbicides. In addition to the data presented by the applicant, the GMO Panel is not aware of any scientific report of increased spread and establishment of soybean A2704-12 and any change in survival capacity, including overwintering. Furthermore there is no evidence that the glufosinate tolerant trait introduced by genetic modification results in increased invasiveness of any crop species, except in the presence of the glufosinate-containing herbicides. The accidental release of soybean A2704-

12 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.

Therefore the GMO Panel is of the opinion that the likelihood of unintended environmental effects of the soybean A2704-12 in Europe will not be different to that of conventional soybean varieties.

5.2.1.2. Potential for gene transfer

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

Based on current scientific knowledge and elaborated in more detail elsewhere (EFSA, 2004), gene transfer from GM plants to microorganisms under natural conditions is extremely unlikely, and its establishment would occur primarily through homologous recombination in microorganisms.

Food and feed products derived from the soybean A2704-12 are likely to contain transgenic DNA. Therefore microorganisms in the digestive tract of humans and animals may be exposed to transgenic DNA.

The *pat* gene is known to be widespread in soil microbial populations. Taking into account the origin and nature of the *pat* gene and the lack of selective pressure for this gene in the intestinal tract, the likelihood that horizontal gene transfer would result in increased fitness of microorganisms is very limited. It is very unlikely that the *pat* gene from soybean A2704-12 would become transferred and established in the genome of microorganisms in the environment (including plant-associated microorganisms *e.g.* rhizobia) or human and animal digestive tract. In the very unlikely event that such a horizontal gene transfer occurs, no adverse effects on human and animal health and the environment are expected as no new traits would be introduced or expressed in microbial communities.

(b) Plant to plant gene transfer

Considering the scope of the application and the physical characteristics of soybean seeds (see section 5.2.1.1), a possible pathway of dispersal is from seed spillage and pollen from the occasional soybean plant originating from accidental seed dispersal during transportation and processing.

The genus *Glycine* is divided into two distinct subgenera: G*lycine* Willd. and *Soja*. Soybean (*Glycine max*) is in the subgenus *Soja*. The subgenus *Glycine* contains 16 perennial species, especially indigenous to Australia, and the subgenus *Soja* contains three annual species, *G. max*, *G. soja*, and *G. gracilis* originally from eastern Asia (Hymowitz *et al.*, 1998; Hymowitz and Singh, 1987). Weedy soybean has not been reported in other parts of the world such as the Americas and Europe where only the cultivated soybean is grown (Lu, 2005). Therefore, the plant to plant gene transfer from this soybean is restricted to cultivated and the occasional soybean plant resulting from seed spillage.

Seed and pollen are potential sources of gene dispersal. 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; Lu, 2005). Soybean pollen dispersal is limited because the anthers mature in bud and directly pollinate the stigma of the same flower (OECD, 2000). However, Ahrent and Caviness (1994) as well as Gumisiriza and Rubaihayo (1978) observed natural cross-pollination rates as high as 2.5 and 4.5%, respectively. Ray *et al.* (2003) recorded natural cross-pollination rates ranging from 0.7 to 6.3%, suggesting the potential of some within-crop gene flow in soybean. These results indicate that natural cross-pollination rates can fluctuate significantly among different soybean varieties under particular environmental conditions such as favourable climate for pollination and abundance of pollinators (Lu, 2005).

The 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 crops and the occasional soybean plant resulting from seed spillage is considered to be extremely low. Even if transgene flow occurred to cultivated soybean plants, a selective advantage would only occur if the complementary glufosinate-containing herbicides were applied.

5.2.1.3. Potential interactions of the GM plant with non-target organisms

This point was not considered an issue by the GMO Panel considering the intended uses of soybean A2704-12, excluding cultivation and consequently the low level of exposure to the environment.

5.2.1.4. Potential interaction with the abiotic environment and biogeochemical cycles

This point was not considered an issue by the Member States or by the GMO Panel. Considering the intended uses of soybean A2704-12, excluding cultivation, the level of exposure to the environment is likely to be extremely low.

5.2.2. Monitoring

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 GMO Panel gives its opinion on the scientific quality of the monitoring plan provided by the applicant (EFSA, 2006a). Exposure to the environment of soybean A2704-12 would be related to accidental release of GM seeds during transportation and processing.

Since the environmental risk assessment does not cover cultivation and identified no potential adverse environmental effects, case-specific monitoring is not considered necessary.

The general surveillance plan proposed by the applicant includes i) the description of an approach involving operators, reporting to the applicants any observed adverse effect of GMOs on human health and the

environment, ii) a coordinating system newly established by EuropaBio, iii) the use of networks of existing surveillance systems. The applicant will submit a general surveillance report on annual basis and a final report at the end of the consent. In case of confirmed adverse effects, the applicant will immediately inform the European Commission and the Member States.

The GMO Panel is of the opinion that the general approaches and measures of the monitoring plan proposed by the applicant are in line with the EFSA opinion on post-market environmental monitoring (EFSA, 2006b) as well as with the intended uses of soybean A2704-12. Since the environmental risk assessment does not cover cultivation and identifies no potential adverse environmental effects, no case-specific monitoring is necessary. The GMO Panel agrees with the proposal made by the applicant on the reporting intervals of the general surveillance plan.

5.3. Conclusion

The scope of application EFSA/GMO/NL/2005/18 is for food and feed uses, import and processing of soybean A2704-12 and excludes cultivation. Considering the proposed uses of soybean A2704-12, there is no requirement for scientific information on potential environmental effects associated with cultivation. The GMO Panel considered the environmental comments raised by Member States in the above sections of Chapter 5. The GMO Panel takes into account that this application does not include cultivation of the soybean so that the likelihood of cross-pollination between cultivated soybean crops and the occasional soybean plant which might occur from accidental release is considered to be extremely low.

If accidental release and subsequent establishment into the environment of soybean A2704-12 plants were to occur, soybean A2704-12 plants would only be fitter in the presence of glufosinate-containing herbicides which are not currently used on cultivated soybean or in most areas where the soybean might be spilled. Therefore the GMO Panel is of the opinion that the likelihood of the spread and establishment of soybean A2704-12 is very low and that unintended environmental effects due to this soybean will be no different from that of conventional soybean varieties. The scope of the monitoring plan provided by the applicant and the reporting intervals are in line with the intended uses of soybean A2704-12 since cultivation is excluded.

CONCLUSIONS AND RECOMMENDATIONS

The GMO Panel was requested to carry out a scientific risk assessment of the soybean A2704-12 for food and feed uses, import and processing.

The GMO Panel is of the opinion that the molecular characterisation of the DNA insert and flanking regions of A2704-12 does not raise safety concerns, and sufficient evidence for the stability of the insert structure and of the newly introduced trait was provided.

Comparative analysis has shown that soybean A2704-12 is compositionally and agronomically equivalent to conventional soybean lines, except for the introduced transgenic trait. The risk assessment included an analysis of data from analytical studies, bioinformatics, and *in vitro* and *in vivo* studies. The GMO Panel concluded that the soybean A2704-12 is as safe as its non GM counterpart and that the overall allergenicity of the whole plant is not changed.

The application EFSA-GMO-NL-2005-18 is for food and feed uses, import and processing. There is therefore no requirement for scientific information on possible environmental effects associated with the cultivation of soybean A2704-12. Considering the scope of the application, not for cultivation, the GMO Panel is of the opinion that the likelihood of the spread and establishment of soybean A2704-12 is very low and that unintended environmental effects due to this soybean will be no different from that of conventional soybean varieties. The scope of the monitoring plan provided by the applicant and the reporting intervals are in line with the intended uses of soybean A2704-12 since cultivation is excluded.

In conclusion, taking into account issues raised by Member States, the GMO Panel considers that, on the basis of the information available for soybean A2704-12, it is unlikely that soybean A2704-12 will have any adverse effect on human and animal health or on the environment in the context of its proposed uses.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Dutch Competent Authority (VROM), dated 12 July 2005, concerning a request for placing on the market of soybean A2704-12 in accordance with Regulation (EC) 1829/2003.

2. Letter from EFSA to applicant, dated 20 December 2005, with request for updated version/additional information (ref. SR/AC/cz(2005) 1477).

3. Letter from applicant to EFSA, dated 31 January 2006, providing an updated version of the application EFSA-GMO-NL-2005-18 submitted by Bayer CropScience under Regulation (EC) 1829/2003:

   Part I – Technical dossier
   Part II – Summary
   Part III – Cartagena Protocol
   Part IV – Labelling and Unique Identifier
   Part V – Samples and Detection
   Part VI – Additional information for GMOs

4. Letter from EFSA to applicant, dated 10 February 2006, delivering the 'Statement of Validity' for application EFSA-GMO-NL-2005-18, soybean A2704-12 submitted by Bayer CropScience under Regulation (EC) 1829/2003 (ref. SR/KL/jq(2006) 1355544).

5. Letter from EFSA to applicant, dated 16 February 2006, with request for additional information (ref. SR/KL/jq(2006) 1375265).

6. Letter from JRC to EFSA, dated 12 May 2006, with complete application (ref. JRC I06-GMO/GVDE/SC/D(2006) (83)11788).

7. Letter from EFSA to applicant, dated 6 July 2006, with request for additional information (ref. SR/LM/jq(2006) 1623108).

8. Letter from applicant to EFSA, dated 31 July 2006, providing additional information upon EFSA request.

9. Letter from EFSA to applicant, dated 24 October 2006, with request for additional information (ref. SR/KL/jq (2006) 1798791).

10. Letter from applicant to EFSA, dated 16 November 2006, providing additional information upon EFSA request.

11. Letter from EFSA to applicant, dated 22 December 2006, with request for additional information (ref. SR/KL/shv(2006) 1894947).

12. Letter from EFSA to applicant, dated 23 January 2007, with request for additional information (ref. SR/CP/shv(2007) 1938116).

13. Letter from applicant to EFSA, dated 26 January 2007, providing additional information upon EFSA request.

14. Letter from EFSA to applicant, dated 23 May 2007, with request for additional information (ref. SR/CP/shv(2007) 2156322).

15. Letter from applicant to EFSA, dated 30 May 2007, providing additional information upon EFSA request.

16. Letter from EFSA to applicant, dated 08 June 2007, with request for additional information (ref. SR/KL/shv(2007) 2183597).

17. Letter from EFSA to applicant, dated 25 June 2007, with request for additional information (ref. SR/CP/shv(2007) 2218520).

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