SCIENTIFIC OPINION

Scientific Opinion on application (EFSA-GMO-UK-2007-43) for the placing on the market of herbicide tolerant genetically modified soybean 356043 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Pioneer[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 356043 for food and feed uses, import and processing. Soybean 356043 contains a single copy of intact *gat4601* and *Glycine max-hra* cassettes at a single insertion locus. The results of the bioinformatic analyses of the insert and the flanking regions, and the levels of newly expressed proteins did not raise a safety concern. The comparative analysis of phenotypic and agronomic characteristics indicated that soybean 356043 is not different from its conventional counterpart. In the composition, differences were identified between 356043 soybean and its conventional counterpart in the newly expressed proteins *Glycine max*-HRA and GAT4601, and the levels of the fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acid and the acetylated amino acids N-acetylaspartate (NAA) and N-acetylglutamate (NAG). The safety assessment of the newly expressed proteins *Glycine max*-HRA and GAT4601 identified no concerns regarding potential toxicity and allergenicity. Heptadecanoic, heptadecenoic and heptadecadienoic acid are present in the diet and the intake of small amounts of these fatty acids via food or feed is not expected to produce adverse effects. NAA and NAG are normal constituents in the mammalian metabolism and the estimated increases in their intake are considered low when related to the normal intake of L-aspartic acid and L-glutamic acid. Further toxicological, allergenicity and nutritional analysis provided no indications of adverse effects. There are no indications of an increased likelihood of establishment and spread of feral soybean plants, except in the presence of the glyphosate and ALS-inhibiting herbicides neither a risk caused by a possible transfer of the recombinant gene from soybean 356043 to environmental micro-organisms. The EFSA GMO Panel considers that the information available for soybean 356043 addresses the scientific comments raised by the Member States and states that the soybean 356043, as

1 On request from the Competent Authority of the UK on an application (EFSA-GMO-UK-20007-43) submitted by Pioneer, Question No EFSA-Q-2007-087, adopted on 6 July 2011.

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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, 356043, risk assessment, food and feed safety, environment, import and processing, Regulation (EC) No 1829/2003, GAT, *Glycine max*-HRA, NAA, NAG.

SUMMARY

Following the submission of an application (EFSA-GMO-UK-2007-43) under Regulation (EC) No 1829/2003 from Pioneer, the EFSA Panel on Genetically Modified Organisms was asked to deliver a scientific opinion on the herbicide tolerant genetically modified (GM) soybean 356043 (Unique identifier DP-356Ø43-5) for food and feed uses, import and processing.

In delivering its scientific opinion, the EFSA GMO Panel considered the application EFSA-GMO-UK-2007-43, additional information supplied by the applicant and scientific comments submitted by Member States. The scope of application EFSA-GMO-UK-2007-43 is for food and feed uses, import and processing of soybean 356043 and all derived products, but excludes cultivation in the EU. The EFSA GMO Panel assessed soybean 356043 with reference to the intended uses and 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 scientific assessment included molecular characterisation of the inserted DNA and expression of target proteins. A comparative analysis of agronomic traits and composition was undertaken, and the safety of the new protein and the whole food/feed were evaluated with respect to potential toxicity, allergenicity and nutritional quality. An assessment of environmental impacts and the post-market environmental monitoring plan were undertaken.

The molecular characterisation data establish that the genetically modified soybean 356043 contains one copy of an intact *gat4601* expression cassette and a *Glycine max-hra* (*gm-hra*) cassette in a single locus. No other parts of the plasmid used for transformation are present in the transformed plant. Bioinformatic analysis of the open reading frames spanning the junctions between the inserted DNA and soybean genomic DNA did not raise safety concerns. The stability of the inserted DNA and the herbicide tolerance trait were confirmed over several generations. Analyses of the levels of newly expressed proteins in various plant tissues collected from field trials performed in South- and North America did not raise safety concerns.

The EFSA GMO Panel concludes that no differences were identified between 356043 soybean and its conventional counterpart, except for the newly expressed proteins, for higher levels of the acetylated amino acids N-acetylaspartate (NAA) and N-acetylglutamate (NAG), and the odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acid in seed from 356043 soybean. The levels of these acetylated amino acids and odd chain fatty acids fall outside the natural ranges observed for other commercial non-GM soybean varieties. The overall level of NAA and NAG (taken together) in soybean 356043 was found to be less than 0.15 % of the total amino acids. The total level of odd chain fatty acids amounts to less than 1% of total fatty acids. No statistically significant differences in total amino acid contents in seed were observed between the 356043 soybean and its conventional counterpart. Levels of major fatty acids in 356043 soybean seed were found to be comparable to those observed in the conventional counterpart.

No toxicity of the GAT4601 and the *Glycine max*-HRA proteins was observed in acute oral toxicity studies and repeated-dose (28 days) feeding studies using mice. The studies on *in vitro* digestibility of the proteins showed that most of the proteins were degraded. In bioinformatics studies the proteins showed no homology to known toxic proteins and allergens.

The odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acid are normal constituents of plants and animals and have also been identified in human tissues. There is no information indicating that the intake of small amounts of these fatty acids via food or feed causes adverse effects. The EFSA GMO Panel is of the opinion that the estimated increases in intake levels of heptadecanoic, heptadecenoic and heptadecadienoic resulting from replacement of conventional soybean oil with oil from soybean 356043 do not raise safety concerns.

NAA and NAG are normal constituents in the mammalian metabolism. They are also present in conventional foodstuffs and thus consumed as part of a normal diet. The available scientific information indicates that under normal conditions NAA and NAG, like other N-acetylated amino acids, are deacetylated in the intestine to form the corresponding L-amino acids, which are further metabolised in the body. The oral toxicity of NAA and NAG has been tested in acute and subacute (28 days) studies using rats. In addition, NAA was tested in a subchronic (90 days) feeding study and in a study on reproductive and developmental toxicity (two generation study) using rats. Considering the outcome of a conservative intake assessment, the estimated increase in intake of NAA is more than 100 fold lower than the NOEL observed in the 90-day rat feeding study with NAA. Furthermore, in relation to the normal intake of L-aspartic acid and L-glutamic acid resulting from consumption of food protein, the estimated increases in the intake of NAA and NAG are considered low. Considering all the available information, the EFSA GMO Panel is of the opinion that the estimated increases in intake levels of NAA and NAG resulting from replacement of food products derived from conventional soybeans by the respective products derived from soybean 356043 do not raise safety concerns. The same conclusion applies to the use of feed materials derived from this genetically modified soybean.

Furthermore, a subchronic 92-day feeding study in rats using diets including meal and hulls derived from soybean 356043 provided no indications of adverse effects. Testing of extracts from soybeans 356043 with sera from patients allergic to soybean showed that the overall allergenicity of the whole plant had not been changed. A 42-day feeding study using broiler chickens demonstrated that soybean 356043 is nutritionally equivalent to its conventional counterpart and commercial non-GM soybean varieties included in this study. Therefore, the EFSA GMO Panel is of the opinion that soybean 356043 is as safe as its conventional counterpart with respect to potential effects on human and animal health in the context of its intended uses.

The application EFSA-GMO-UK-2007-43 is for food and feed uses, import and processing. Therefore, there is no requirement for scientific information on possible environmental effects associated with the cultivation of soybean 356043. 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 356043 (e.g. during transportation and processing), except in the presence of glyphosate and ALS-inhibiting 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. In the context of its intended uses, the theoretically possible transfer of the recombinant genes from soybean 356043 to gut or other environmental bacteria has not been identified to be a risk due to the lack of any selective advantage. 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 356043 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 356043 addresses the scientific comments raised by the Member States and that the soybean 356043, 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 11 April 2007, the European Food Safety Authority (EFSA) received from the Competent Authority of the United Kingdom an application (Reference EFSA-GMO-UK-2007-43), for authorisation of the herbicide tolerant genetically modified (GM) soybean 356043 (Unique Identifier DP-356Ø43-5), submitted by Pioneer within the framework of Regulation (EC) No 1829/2003 on genetically modified food and feed. After receiving the application EFSA-GMO-UK-2007-43 and in accordance with Articles 5(2)(b) and 17(2)(b) of Regulation (EC) No 1829/2003, EFSA informed Member States and the European Commission, and made the summary of the application publicly available on the EFSA website. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of Regulation (EC) No 1829/2003. On 11 September 2007, EFSA received additional information requested under completeness check (requested on 06 August 2007). On 28 September 2007, 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 Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Member State bodies had three months after the date of acknowledgement of the valid application (28 December 2007) within which to make their opinion known.

The Scientific Panel on Genetically Modified Organisms of EFSA (EFSA GMO Panel) carried out a scientific assessment of the GM soybean 356043 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. When carrying out the safety assessment, the EFSA GMO Panel took into account 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 scientific comments of Member States and the additional information provided by the applicant.

On 20/12/2007, 27/02/2008, 22/07/2008, 08/09/2008, 14/01/2010, 28/05/2010 and 22/10/2010 the EFSA GMO Panel requested from the applicant additional information. The applicant provided the requested information on 12/02/2008, 15/04/2008, 06/10/2009, 08/09/2008, 15/10/2008, 05/03/2010, 12/07/2010 and 07/12/2010. Complementary information was submitted spontaneously by the applicant on 21/04/2010 and 11/03/2011. After receipt and assessment of the full data package the EFSA GMO Panel finalised its risk assessment on soybean 356043.

In giving its scientific opinion on GM soybean 356043 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 six 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 risk assessment of soybean 356043 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 356043 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 356043 (Unique Identifier DP-356Ø43-5) 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 the 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 the relevant scientific data

3.1.1. Transformation process and vector constructs[5]

The 356043 soybean has been genetically modified for herbicide tolerance. This was achieved by the introduction of the *gat4601* and the *Gycine max-hra* (*gm-hra*) coding sequences surrounded by their necessary regulatory components.

- *gat4601* is an optimized form of the glyphosate acetyltransferase *(gat)* coding sequence from *Bacillus licheniformis* that confers tolerance to glyphosate- and glyphosate-ammonium based herbicides. Glyphosate inhibits the enzyme enolpyruvulshikimate-3-phosphate synthase (EPSPS), which is involved in the biosynthesis of aromatic amino acids. GAT proteins acetylate glyphosate giving rise to N-acetyl glyphosate, which has no herbicidal activity. The synthetic *gat4601* coding sequence was obtained after seven rounds of DNA shuffling using three distinct alleles of the *gat* gene isolated from three different strains of *B. licheniformis* as well as the introduction of changes *via* PCR. The native GAT enzymes were capable of acetylating glyphosate, but at a very slow rate. The GAT4601 protein is 84% homologous at the amino acid level to each of the three GAT enzymes from *B. licheniformis* from which it was derived but with 2400-fold increased catalytic efficiency. The GAT proteins are members of the GNAT family of N-acetyltransferases. GNAT proteins have a number of metabolic functions (Dyda et al. 2000). The studies on substrate specificity of GAT4601 concluded that it acetylates aspartic and glutamic acids, and has very low affinity to serine, threonine, glycine and some aminophosphonates.

- *gm-hra* is an optimized form of the endogenous acetolactate synthase (*als*) coding sequence from soybean (*Glycine max*), that confers tolerance to ALS-inhibiting herbicides, such as chlorimuron, thifensulfuron or sulfonylureas. The synthetic *gm-hra* coding sequence was obtained by introducing a synthetic start codon together with twelve nucleotides from the endogenous *als* 5' untranslated region at the 5' end of the endogenous *als* gene. In addition, two nucleotide changes were made within the coding sequence with the purpose of introducing two point mutations (A183 and L560) in the protein sequence. As a result, the *Glycine max*-HRA protein is tolerant to ALS-inhibiting herbicides.

[4] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2011-00856
[5] Technical Dossier / Sections C and D1

The 356043 soybean was produced by the particle acceleration method. A linear DNA fragment containing the *gat4601* and *gm-hra* expression units was inserted into soybean plant cells from the commercial cultivar "Jack". The DNA fragment (PHP20163A) introduced into 356043 soybean was obtained from plasmid PHP20163 following digestion of the plasmid DNA with restriction enzymes. PHP20163A contains the *gat4601* and *gm-hra* expression units in tandem orientation.

- The *gat4601* coding sequence is under the regulation of the synthetic constitutive promoter (SCP1) comprising a portion of the *Cauliflower mosaic virus* 35S promoter and the Rsyn7-Syn II core consensus promoter, with translation enhanced by omega 5' untranslated region translational enhancer element from the *Tobacco mosaic virus*, and with transcription terminated by the proteinase inhibitor II (*pin*II) terminator from potato (*Solanum tuberosum*).

- The *gm-hra* coding sequence is under the regulation of the constitutive S-adenosyl-L-methionine synthetase (SAMS) promoter from soybean, and with transcription terminated by the endogenous *als* gene terminator from soybean.

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

Molecular analyses were undertaken on T_4 plants produced after four generations of self-pollination of the original transformation event DP-356043-5. Southern blot, PCR, sequencing and inheritance studies established that a single, intact PHP20163A fragment was inserted into the soybean nuclear genome to produce 356043 soybean. The absence of additional DNA sequences from the PHP20163 plasmid in 356043 plants has been confirmed by Southern analysis using probes that cover the entire sequence of the plasmid backbone (including the hygromycin resistance gene used to maintain the plasmid in bacteria).

The DNA sequence of the insert contains 5362 base pairs spanning the entire PHP20163A fragment. Flanking genomic sequences extending 3317 base pairs at the 5' end and 2169 base pairs at the 3' end of the insert were determined. Both flanking regions were shown to be soybean genomic sequences. The applicant carried out further bioinformatics analysis (BLASTn, BLASTx) in order to identify the nature and potential function of the soybean flanking sequences[7]. The outcome was that genetic modification did not interrupt any known genes.

The applicant performed a bioinformatic analysis of all twelve open reading frames spanning the insert – genomic DNA junction regions in order to assess the similarity of their putative translational products to known toxins and allergens[7,8]. No similarities were found.

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

The expression levels of GAT4601 and *Glycine max*-HRA were measured by ELISA in several samples of 356043 soybean cultivated in field trials at six locations during one season in South America (2005/2006) and at one location during one season in North America (2005). The expression levels were determined from forage, root and grain at different growth stages and from plants treated and non-treated with herbicides. The expression level of GAT4601 varied between 0.09 and 1.0 µg/g dry weight (dw)10 for grain and between 0.72 and 2.3 µg/g dw[11] for forage. The *Glycine max*-HRA expression level ranged between the level of detection and 1.2 µg/g dw10 for grain and between 5.6 and 46 µg/g dw11 for forage. Mean levels of the newly expressed proteins in treated and non-treated

[6] Technical Dossier / Section D2
[7] Additional information, April 2008
[8] Additional information, October 2008
[9] Technical Dossier / Section D3
[10] Technical Dossier, Tables 4-5
[11] Technical Dossier, Annexes 4-5

plants were very similar. The expression ranges of the newly expressed proteins are summarised in Table 1.

Table 1: Ranges of GAT4601 and *Glycine max*-HRA levels in soybean 356043 (µg/g dw)

Site / season	tissue	GAT4601	*Glycine max*-HRA
South America (2005/2006)	grain forage	0.09 – 0.43 0.72 – 1.9	< LOD – 1.1 5.6 – 34
North America (2005)	grain forage	0.12 – 1.2 1.1 – 2.3	< LOD – 1.2 11 – 46

LOD: limit of detection

3.1.4. Inheritance and stability of inserted DNA[12]

Genetic stability of 356043 soybean was investigated by Southern and Western analyses in two populations of plants, one segregating (F_3) and one not segregating (T_5) for the insert. Southern analysis of 92 F_3 individual plants spanning both 5' and 3' on the insert showed that the insert was genetically stable and followed the Mendelian inheritance pattern of a single locus. A further study across two generations (T_4-T_5) confirmed the genetic and phenotypic stablility of the insert. The EFSA GMO Panel is of the opinion that, should instability leading to loss of the trait(s) occur, no safety concern would arise.

3.2. Conclusion

Appropriate molecular and bioinformatic analyses of the 356043 soybean insert and its flanking genomic regions have been undertaken. The expression of the genes introduced 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 356043 soybean is sufficient for the safety assessment. The GMO panel considers this to be an adequate analysis and the molecular characterisation 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[13]

In the compositional studies, the 356043 soybean was in replicated field trials compared to the non-transgenic variety Jack, which is a conventional soybean variety with a history of safe use and with background genetics similar to 356043 soybean. The 356043 soybean and its conventional counterpart were grown under the same agronomic conditions. In addition plots were included where 356043 soybean was treated with glyphosate herbicides and/or ALS inhibiting herbicides. The field trials were in the season 2005-2006 carried out in Chile and Argentina and in year 2005 in USA and Canada, each season/year at six different geographical sites. Additional field trials for agronomic and compositional analyses were performed at four locations in the USA and two locations in Canada in 2006. Five of the six locations in North America were planted both in 2005 and 2006. Data obtained for 356043 soybean and its conventional counterpart were compared to ranges for agronomic and compositional characteristics obtained from other commercial non-GM soybean varieties. Data to define natural ranges were derived from literature and from data collected under a separate study, in which four commercially available soybean varieties grown at six locations in North America (2005) were planted, harvested, processed, and analyzed using the same methods employed in the comparative

[12] Technical Dossier / Section D5
[13] Technical dossier/ Section D7.1 and D7.2 and additional information, April 2008 and March 2010

analysis of 356043 soybean. All the locations used in the field trials over the three seasons are representative for the environmental conditions of commercial soybean production in North and South America.

4.1.2. Compositional analysis[14]

Soybean seeds were analysed for proximates, fibre fractions, minerals, amino acids, fatty acids, vitamins, anti-nutrients (i.e. phytic acid, trypsin inhibitor, lectins, stachyose and raffinose) and other secondary metabolites (isoflavones). Forage was analysed for proximates, including fibre fractions. The selection of compounds followed the recommendations by OECD (2000). In addition, compounds related to the activities of the proteins newly expressed in 356043 soybean were analysed in soybean seeds, i.e. acetylated amino acids, free amino acids, and minor odd chain fatty acids. The data on each analyte were statistically analysed for potential differences in their levels in 356043 soybean compared to those in its conventional counterpart within-site and across-sites (data from all sites combined).

Consistent statistically significant compositional differences between 356043 soybean and its conventional counterpart were found for the odd chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acid, independently of the herbicide treatment regime. Levels determined for 356043 soybean were around two to three times higher than those observed for the conventional counterpart and outside the ranges observed for other commercial soybean varieties (Table 2). The range for heptadecadienoic acid in commercial soybeans was determined by the applicant by analysis of material obtained from eight soybean varieties grown in three field studies in the US and Canada (2007, 2009).

Table 2: Levels [% of total fatty acids] of heptadecanoic acid (C17:0), heptadecenoic acid (C17:1) and heptadecadienoic acid (C17:2) in seeds from 356043 soybean untreated or treated with glyphosate and ALS-inhibiting herbicides (i.e. target herbicides) compared to those in seeds from the conventional counterpart Jack (North American locations, 2006)

Analyte		Control soybean Jack untreated with target herbicides	356043 soybean untreated with target herbicides	356043 soybean treated with target herbicides	Reference varieties
C17:0	**Mean**	0.129	0.326	0.330	0.085 - 0.146
	Range	0.105 - 0.304	0.207 - 0.408	0.152 - 0.423	
C17:1	**Mean**	0.063	0.179	0.183	0.073 - 0.087
	Range	0.049 - 0.136	0.117 - 0.240	0.067 - 0.248	
C17:2	**Mean**	0.056	0.150	0.153	0 - 0.068
	Range	0.045 - 0.121	0.099 - 0.203	0.061 - 0.211	

As an explanation for this effect the applicant considered that odd chain fatty acid biosynthesis starts with the conversion of 2-ketobutyrate to propionyl-CoA followed by subsequent addition of C2 moieties. One of the specific amino acid changes introduced into the *Glycine max*-ALS enzyme to form the *Glycine max*-HRA enzyme conferring herbicide tolerance (i.e. replacement of tryptophan 560 by leucine), is expected to increase the 2-ketobutyrate pool available for odd chain fatty acid biosynthesis due to decreased affinity to that intermediate. Studies on the odd chain fatty acids showed increased levels of the C17 long fatty acids but the contents of the longer odd chain fatty acids C19:0, C21:0 and C23:0 have not been altered in 356043 soybean seeds and that they are comparable to the levels in the conventional counterpart. Statistically significant differences occasionally observed for other fatty acids were considered to be small and not biologically relevant. Levels of major fatty acids

[14] Technical dossier/ Section D7.2 and D7.3 and additional information of April 2008, September 2008, March 2010 and December 2010

in 356043 soybean seeds were found to be comparable to those observed in the conventional counterpart.
In tests for substrate specificity, the newly expressed GAT4601 protein was shown to acetylate aspartic acid and glutamic acid. The protein was found to have a very low affinity for serine, threonine and glycine. (see section 5.1.3.2.). The levels of the acetylated amino acids N-acetylaspartate (NAA) and N-acetylglutamate (NAG) were measured in seeds of 356043 soybean, its conventional counterpart and commercial soybean varieties. The mean values for NAA and NAG in 356043 soybean were consistently statistically significantly different from those of its conventional counterpart and markedly outside natural ranges determined for commercial soybean varieties. This effect was observed independently of the herbicide treatment regime (Table 3).

Table 3: Levels [mg/kg dry weight] of N-acetylaspartate (NAA) and N-acetylglutamate (NAG) in seeds from 356043 soybean untreated or treated with glyphosate and ALS-inhibiting herbicides (i.e. target herbicides) compared to seeds from control soybean (South American locations, 2005-2006)

Analyte		**Control soybean Jack untreated with target herbicides**	**356043 soybean untreated with target herbicides**	**356043 soybean treated with target herbicides**	**Reference varieties**
NAA	**Mean**	1.92	653	681	0 - 2.27
	Range	1.10 - 3.67	490 - 870	502 - 994	
NAG	**Mean**	2.34	18.3	18.1	0 - 3.17
	Range	1.42 - 3.35	9.86 - 43.2	8.27 - 31.8	

The applicant was requested by the GMO Panel to quantify acetylated derivatives of serine, threonine and glycine in 356043 soybean and its conventional counterpart. It was demonstrated that the levels in seed from 356043 soybean and its conventional counterpart are comparable and within natural ranges calculated for these compounds in non-genetically modified soybean seed.

Considering the modes of action of the GAT4601 and *Glycine max*-HRA proteins newly expressed in 356043 soybean, comprehensive comparative analyses of total and free amino acids were carried out. No statistically significant differences in total amino acid contents in seed were observed between the 356043 soybean and its conventional counterpart for any of the eighteen proteinogenic amino acids tested. In addition, the levels of free amino acids in seed from 356043 soybeans are comparable to the levels of free amino acids in seed from its conventional counterpart, regardless of the treatment with glyphosate and ALS-inhibiting herbicides. The overall level of NAA and NAG (taken together) in soybean 356043 was found to be less than 0.15 % of the total amino acids.

For other key constituents, recommended by OECD, including anti-nutrients and other secondary metabolites (isoflavones), no consistent alteration in the level of the studied components in 356043 soybeans as compared to the conventional counterpart was found between sites and between growing seasons. Furthermore, the differences were generally small and fell within the range of natural variation calculated from the occurrence of these constituents in other commercial soybean varieties.

Compositional analysis of soybean forage did not reveal consistent alterations in the level of studied components in 356043 soybean as compared to the conventional counterpart. The composition of forage obtained from 356043 soybean fell within the range of natural variation. The applicant did not provide information on the levels of acetylated amino acids in forage.

The Panel considered the total compositional data supplied and the observed compositional differences between 356043 soybean and its conventional counterpart in the light of the measured biological variation and the level of the studied compounds in other commercial non-GM soybean varieties. The EFSA GMO Panel concludes that no differences were identified between 356043 soybean and its conventional counterpart, except for the newly expressed proteins, for higher levels of the acetylated

amino acids N-acetylaspartate (NAA) and N-acetylglutamate (NAG), and the odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acid in seed from 356043 soybean. The levels of these acetylated amino acids and odd chain fatty acids fall outside the natural ranges observed for other commercial non-GM soybean varieties.

4.1.3. Agronomic traits and GM phenotype[15]

The applicant provided information on agronomic performance, phenotypic characteristics and ecological interaction of 356043 soybean and its conventional counterpart from field trials performed in the USA, Canada, Chile and Argentina in 2005 and 2006. The characteristics evaluated were early population, final population, seedling vigour, lodging, shattering, disease incidence, insect damage, plant height, days to maturity, yield, flower colour, pod wall colour, and hila colour. When analysed across locations, statistically significant differences were observed for some agronomic parameters, i.e. lodging, seedling vigour, final population, and plant height. However, when analysed by site, statistically significant differences for seedling vigour and plant height were observed at one and four of the six locations situated in North America, respectively. Statistically significant differences were observed at one of the six locations in the individual location analysis for both lodging and plant height in South America. As the magnitudes of the differences were small, and parameters fell within the ranges observed for conventional soybean, the GMO Panel found these differences to be of no biological relevance.

The EFSA GMO Panel assessed the provided data and considers 356043 soybean to be agronomically not different from its conventional counterpart with the exception of the newly introduced traits.

4.2. Conclusion

The EFSA GMO Panel concludes that no differences were identified between 356043 soybean and its conventional counterpart, except for the newly expressed proteins, for higher levels of the acetylated amino acids N-acetylaspartate (NAA) and N-acetylglutamate (NAG), and the odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acid in seed from 356043 soybean. The levels of these acetylated amino acids and odd chain fatty acids fall outside the natural ranges observed for other commercial non-GM soybean varieties. The overall level of NAA and NAG (taken together) in soybean 356043 was found to be less than 0.15% of the total amino acids. The total level of odd chain fatty acids amounts to less than 1% of total fatty acids. No statistically significant differences in total amino acid contents in seed were observed between the 356043 soybean and its conventional counterpart. Levels of major fatty acids in 356043 soybean seed were found to be comparable to those observed in the conventional counterpart. The observed differences are further evaluated in the following Food/Feed safety assessment (section 5).

5. Food/Feed safety assessment

5.1. Evaluation of relevant scientific data

5.1.1. Product description and intended use

The scope of application EFSA-GMO-UK-2007-43 is for food and feed uses, import and processing of soybean 356043. Thus soybean 356043 will be used for the production of soybean products as any commercial soybean variety. The main product for human use is soybean oil. In addition, soybean is used for the production of soybean milk, protein isolate, flour, sprouts, baked or roasted soybeans, tofu, soybean sauce and other products for human consumption. Defatted soybean meal is used as a source of protein in animal feed, often in combination with soybean hulls. There is also a limited direct use of soybeans an animal feed.

[15] Technical dossier/ Section D7.4

The genetic modification of soybean 356043 is intended to improve agronomic performance only and is not intended to influence the nutritional properties, processing characteristics and overall use of soybean as a crop.

5.1.2. Effect of processing[16]

The applicant has studied the influence of temperature (36–60°C) and pH-value (5–9) on the enzyme activity of the *Glycine max*-HRA protein produced in *Escherichia coli* (see 5.1.3.1.) using an ALS activity assay based on the production of acetolactate from pyruvate. After incubation at 44°C for 15 minutes approximately 50% of the activity was lost, and the enzyme was practically inactivated after incubation at 50°C for 15 minutes. The pH optimum of the enzyme activity was in the range of pH 7–7.5, whereas there was practically no activity at or below pH 6.0 as well as at pH 9.0.

The influence of temperature (36–60°C) and pH-value (5–9) on the enzyme activity of the GAT4601 protein produced in *E. coli* (see 5.1.3.1.) was studied using a glyphosate acetyltransferase assay. After incubation at 50 °C for 15 minutes approximately 40% of the activity was lost, and the enzyme was practically inactivated after incubation at 56 °C for 15 minutes. The pH optimum of the enzyme activity was in the range of pH 6–6.5, whereas the activity was considerably reduced at pH 5 and pH 8.5.

Considering the significant compositional differences observed for the raw agricultural commodity (see section 4.1.2), the applicant has provided data on the levels of the acetylated amino acids NAA and NAG, determined by HPLC/MS in whole soybeans and processed products derived from soybean 356043 (untreated and treated with the target herbicides) and its conventional counterpart (Jack).

Compared with the conventional counterpart higher levels of NAA and NAG were present in whole cooked seed, hull material, defatted raw flakes, defatted toasted meal, mill feed, defatted flour, and soy milk from soybean 356043. Higher levels of NAA only, were found in aspirated seeds fraction, crude lecithin, protein concentrate, okara and tofu. NAA and NAG were not detected or below the limit of quantification in protein isolate, and degummed and refined/bleached/deodorised soybean oil.

In processed products the levels of these acetylated amino acids were generally reduced or in the same ranges as in whole unprocessed soybeans except for higher levels in hull material and mill feed (NAA and NAG), defatted raw flakes and defatted toasted meal (slightly higher levels of NAG only).

In summary, processing of whole 356043 soybeans can lead to lower as well as higher levels of the acetylated amino acids NAA and NAG in processed products compared with unprocessed 356043 soybeans.

Since soybean oil is the primary source of human exposure, the applicant determined the compositions of crude and refined bleached deodorized soybean oil obtained from 356043 soybean (treated and untreated with target herbicides) and its conventional counterpart. Soybean samples were collected from six separate field trials in 2006, four located in the United States and two located in Canada. Oil from 356043 soybean differed from oil from its conventional counterpart in the levels of heptadecanoic and heptadecenoic acid. Results obtained for oil were fully in line with those determined for seeds. Upon request of the EFSA GMO Panel to assess the potential intake of the odd-chain fatty acids, the applicant also provided information on the levels of heptadecadienoic acid in refined bleached deodorized soybean oil derived from 356043 soybeans and its conventional counterpart (see section 5.1.5.3).

[16] Technical dossier/ Section D7.6 and additional information April 2008

5.1.3. Toxicology[17]

5.1.3.1. Proteins used for safety assessment

Given the low levels of the proteins GAT4601 and *Glycine max*-HRA expressed in soybean 356043 and the difficult task to isolate a sufficient quantity of purified proteins from this soybean, proteins produced in recombinant *Escherichia coli* strains were used for the safety testing.

The equivalence of the GAT4601 protein produced in *E. coli* to that produced in leaf tissue of soybean 356043 was shown by SDS-PAGE, Western analysis, MALDI-MS analysis of tryptic peptides, N-terminal amino acid sequence analysis and glycosylation analysis. The identity of the microbial protein was further confirmed using electrospray mass spectroscopy, amino acid composition analysis and an enzyme activity assay.

In the case of the *Glycine max*-HRA protein the mature form (604 amino acids), which does not contain the chloroplast transit peptide that is cleaved from the protein during processing in the plant, was produced in *E. coli* in the form of a fusion protein. Due to the cleavage of this fusion protein with thrombin during the purification process, the resulting microbial *Glycine max*-HRA protein has an additional glycine residue at the N-terminus compared to the *Glycine max*-HRA protein expressed in soybean 356043. The equivalence of the *Glycine max*-HRA protein produced in *E. coli* to that produced in leaf tissue of soybean 356043 was shown by Western analysis, N-terminal amino acid sequence analysis, MALDI-MS analysis of tryptic peptides and glycosylation analysis. In addition, the identity of the microbial protein was corroborated using electrospray mass spectroscopy, analysis of the amino acid composition and determination of the enzyme activity.

The EFSA GMO Panel therefore accepts the test materials derived from *E. coli* as appropriate substitute test materials for the GAT4601 and *Glycine max*-HRA proteins present in soybean 356043 in the safety studies.

5.1.3.2. Toxicological assessment of expressed novel proteins in soybean 356043[18]

The *Glycine max*-HRA protein expressed in soybean 356043 is an acetolactate synthase (ALS) encoded by a modified *als* gene from soybean (*Glycine max*). ALS enzymes are key enzymes in the biosynthesis of the essential branched-chain amino acids, where they catalyse the first common step in the biosynthesis of isoleucine, leucine and valine starting from pyruvate (LaRossa and Falco, 1984; Duggleby and Pang, 2000). The enzyme catalyses two reactions, these being the conversion of two molecules of pyruvate into 2-acetolactate, used in the synthesis of leucine and valine, and the condensation of pyruvate with 2-ketobutyrate producing 2-acetohydroxybutyrate, used in the synthesis of isoleucine. ALS enzymes are widespread in nature, and occur e.g. in plants, algae, yeast and bacteria (Friden et al., 1985; Falco and Dumas, 1985; Mazur et al., 1987; Mazur and Falco, 1989; Reith and Mulholland, 1995). The *Glycine max*-HRA protein (656 amino acids) expressed in soybean 356043 is a modified version of the endogenous ALS precursor protein. Compared with the endogenous ALS precursor protein in soybean, it includes 5 additional amino acid residues at the N-terminus and two internal amino acid changes. The amino acid sequence of the mature form (after cleavage of the chloroplast transit peptide) differs from that of the mature endogenous soybean protein in two out of 604 amino acids (see section 3.2.1). These changes confer tolerance to ALS-inhibiting herbicides to the modified soybean.

The GAT4601 protein (146 amino acids, molecular mass ca. 17 kDa) is an optimised form of the enzyme glyphosate acetyltransferase (GAT) from *Bacillus licheniformis*, which acetylates glyphosate using acetyl-CoA as acetyl-donor. The coding sequence was obtained after seven rounds of DNA shuffling using three distinct alleles of the *gat* gene isolated from three different strains of *B. licheniformis* (see section 3.2.1.). The GAT4601 protein shows an 84% sequence homology at the

[17] Technical dossier/ Section D7.8
[18] Technical dossier/ Section 7.8.1/additional information April 2008 and September 2008

amino acid level to each of the three GAT enzymes from *B. licheniformis* from which it was derived and has an increased catalytic efficiency. The enzymatic aceytlation of glyphosate produces N-acetylglyphosate and renders the plant tolerant to glyphosate herbicides. The GAT proteins are members of the GCN5-related family of N-acetyltransferases (GNAT family), consisting of more than 10 000 representatives from all kingdoms of life. Members of the GNAT family contain a highly conserved GNAT motif but show high sequence diversity in other parts of the protein and have diverse functions (Vetting et al., 2005; Dyda et al., 2000).

The substrate specificity of the GAT4601 protein was studied *in vitro* using 21 amino acids, 11 antibiotics and 20 different agrochemicals. In these studies GAT4601 acetylated aspartic acid and glutamic acid with relatively low efficiency compared with the acetylation of glyphosate. The affinity of the protein for serine, threonine and glycine was so low that a K_M value could not be estimated. The enzyme did not show detectable activity on the other tested substances.

(a) Acute toxicity testing[19]

The proteins GAT4601 and *Glycine max*-HRA produced in *E. coli* were tested separately for acute oral toxicity using mice and did not induce adverse effects after administration of single doses of 1596 and 582 mg/kg bw, respectively.

(b) Repeated-dose testing[20]

The applicant provided a repeated-dose feeding study using the GAT4601 protein produced in *E. coli* as test material. Groups of 5 male and 5 female mice (CD-1) received the GAT4601 protein at dietary doses of 7.8, 76.7 or 783.1 mg/kg bw/day (males) and 9.2, 94.4 or 926.9 mg/kg bw/day (females) for 27 days. The diets were not adjusted for protein content, and the control group received a standard rodent diet without additional protein. Throughout the treatment period there was no mortality, and the regular observations of the animals revealed no clinically relevant findings that were considered related to the test material. There were no relevant differences in feed consumption between the groups and no statistically significant changes in mean body weights (except for one value at day 1 of the treatment period) as well as body weight gains compared with the control group. In ophthalmic examinations no abnormalities were noted. In haematology examinations no statistical significant differences compared with the control group were observed. Clinical-chemistry analyses showed statistically significantly lower levels of plasma total protein and albumin in females of the high-dose group compared with the control group. The differences were small and the mean values fell within the ranges of the historical controls. In the absence of differences in related parameters indicating liver or kidney toxicity these differences, which were not observed in male animals, are not considered toxicologically relevant. Males of the low and high-dose groups had lower plasma potassium levels. However, this effect was not dose-related and no differences in the plasma levels of other electrolytes were observed. Therefore the differences are considered as incidental. Determination of the weights of selected organs and tissues did not reveal statistically significant differences except for reduced mean absolute spleen weight as well as spleen weight in relation to brain weight but not in relation to body weight in females of the low- and high-dose groups. Furthermore microscopic examinations of organs and tissues, including the spleen, revealed no gross lesions and no relevant differences in microscopic findings between the groups. The EFSA GMO Panel concludes that there were no indications of adverse effects up to the highest dose tested.

On request of the EFSA GMO Panel the applicant provided the complete report on a repeated-dose feeding study using the *Glycine max*-HRA protein[21], which was described in a scientific publication (Mathesius et al., 2009). Groups of 5 male and 5 female mice (CD-1) were fed diets containing the *Glycine max*-HRA protein produced in *E. coli* with the diet for 27 days. The actual doses administered

[19] Additional information of March 2010
[19] Additional information of September 2008 and March 2010
[21] Additional information July 2010

were 107.4, 301.8 and 991.7 mg/kg bw/day for males and 123.2, 382.6 and 1247.1 mg/kg bw/day for females. One control group received a standard rodent diet, and another was fed a diet containing bovine serum albumin (BSA) at a dose of 1066.4 mg/kg bw/day (males) and 1337.0 mg/kg bw/day (females). The study was not carried out according to OECD guideline 407 since haematology data were not provided. No mortality occurred during the treatment period and general clinical condition, performance in functional observational tests and motor activity measurements were not affected by the treatment. Ophthalmoscopy results were unremarkable. Feed consumption and body weight development were comparable in all groups. Clinical-chemistry analyses showed no statistically significant differences in groups fed diets with the *Glycine max*-HRA protein compared with the control groups. Organ weight determinations showed statistically significantly lower adrenal weights (absolute and in relation to body weight) in male animals of the mid-dose group only, which was thus considered as an incidental finding. No difference in spleen weight was observed in female animals. Statistically significantly lower mean spleen weights (absolute and in relation to body weight) were observed in males of the medium and high dose groups. The absence of a dose effect relationship as well as the absence of relevant findings in the histopathological examinations of this organ and other tissues of the reticulo-endothelial system suggests that the difference in spleen weight in males is not a result of *Glycine max*-HRA treatment. Macroscopic and histopathological examinations at necropsy did not reveal relevant changes in the other organs and tissues examined. The available results do not indicate adverse effects.

(c) Degradation in simulated digestive fluids

The digestibility of the proteins GAT4601 and *Glycine max*-HRA produced in *E. coli* were studied *in vitro* using pepsin-containing simulated gastric fluid (SGF) and pancreatin-containing intestinal fluid (SIF).

After incubation of the protein *Glycine max*-HRA in SGF (pH 1.2) for 30 seconds no intact protein was detected using SDS-PAGE and protein staining. At this time point a faint band (ca. 3 kDa) was visible, which further decreased in intensity until the last time point in this study (60 minutes). After incubation of the protein *Glycine max*-HRA in SIF (pH 7.5) no intact protein was detectable after 30 seconds using SDS-PAGE and protein staining. Using Western analysis intact protein was still detectable after 30 seconds but not after 1 minute. Several bands, which probably represent degradation products of the *Glycine max*-HRA protein were detected after 30 seconds and 1 minute but not at later time points.

No intact protein was detected by SDS-PAGE and protein staining after incubation of the GAT4601 protein in SGF for 30 seconds. A faint band (ca. 3 kDa) was visible, which was still detectable after 60 minutes of incubation. After incubation in SIF for 30 seconds and 1 minute intact GAT4601 protein was still detectable by SDS-PAGE and protein staining. Using Western analysis the intact protein was detectable after incubation for up to 2 minutes, but at later time points, neither the intact protein nor fragments were detectable.

The *in vitro* digestion experiments demonstrated that the proteins *Glycine max*-HRA and GAT4601 are degraded by digestive enzymes.

(d) Bioinformatic studies[22]

Bioinformatics-supported comparison of the amino acid sequence of the protein GAT4604 with the sequences stored in a general protein sequence database, revealed homology of the GAT4604 protein with other acetyltransferases but not with known toxic proteins.

[22] Additional information April 2008

Analysis of the amino acid sequence of the *Glycine max*-HRA precursor protein showed homology to related acetolactate synthase proteins (ALS) and acetohydroxyacid synthase (AHAS) proteins as well as other functionally related proteins, but not with known toxic proteins.

5.1.3.3. Toxicological assessment of changed levels in natural constituents [23]

The comparative compositional analysis has shown that in seeds from soybean 356043 the levels of the acetylated amino acids N-acetylaspartate (NAA) and N-acetylglutamate (NAG) as well as the levels of the odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acid are higher than in seeds derived from the conventional counterpart and from other commercial soybean varieties (see section 4.1.2).

5.1.3.4. Information on NAA and NAG

NAA and NAG are normal constituents in the mammalian metabolism. N-acetylation is a widespread process in metabolism and is mediated by a number of both specific and unspecific N-acetyltransferases.

NAA is synthesised from acetyl-CoA and L-aspartic acid by acetyl-CoA_L-aspartate N-acetyltransferase (E.C. 2.3.1.2) in neurons only. NAA is the second most abundant free amino acid in brain after glutamate (Tallan et al., 1956, Miyake et al., 1981, Alonso et al., 19991, Tsai and Coyle, 1995). After transport to oligodendrocytes NAA is split into aspartate and acetate by the enzyme aspartoacylase or aminoacylase-2 (E.C.3.5.1.15) which preferentially hydrolyses NAA. In contrast, all other acetylated amino acids are cleaved by aminoacylase-1 (EC 3.5.1.14). The functions of NAA in the central nervous system are still under investigation but include the provision of acetate for myelin lipid and for steroid synthesis, and a precursor role for the neuron specific dipeptide N-acetylaspartylglutamate (NAAG), the most concentrated neuropeptide in human brain which when split by NAAG peptidase delivers NAA. It was shown that intraperitoneally injected NAA does not reach the central nervous system (Berlinguet and Laliberté, 1965).

Low concentrations of NAA were also detected in other organs, e.g. the liver and kidneys. Ingested N-acetylated amino acids are presumably deacetylated by aminoacylase-1 the most abundant of the aminoacylases and expressed in all nucleated human cells, including the intestine and the kidneys, which means that under normal conditions acetylated amino acids are not absorbed in the gut or excreted in the urine to great extent. Deficiency of aminoacylase-1, an inborn error of metabolism, is characterised by considerable urinary excretion of several acetylated amino acids (Sass et al., 2006). Studies in mice using radiolabelled NAA and L-aspartic acid showed that after intraperitoneal injection both substances were metabolised at a similar rate (as determined by measurement of expired radioactive CO_2) indicating a rapid hydrolysis of the N-acetyl group.

NAG is intramitochondrially produced from L-glutamate by N-acetylglutamate synthase (NAGS) using acetyl-CoA. High concentrations of this enzyme are present in the liver and the epithelial cells of the small intestine (Uchiyama et al., 1981; Caldovic et al., 2002 a and b) and correspondingly, high concentrations of NAG were found in these tissues (Shigesada and Tatibana, 1971). NAG is an obligatory allosteric activator of mitochondrial carbamoyl phosphate synthase I (CPSI) (Hall et al., 1958; Caldovic and Tuchmann, 2003), the rate-limiting first step in the mammalian urea cycle. Intramitochondrially formed NAG is transported into the cytosol where it is cleaved by aminoacylase. Mitochondrial uptake of cytosolic NAG is not possible. Ingested NAG will be deacetylated like NAA by aminoacylase-1 and studies in rats, dogs and pigs with orally, enterally or parenterally administered N-acetylglutamine as a substitute for glutamine have shown that the nutritional value of N-acetylglutamine was comparable to that of L-glutamine (Neuhäuser-Berthold, et al., 1988; Gouttebel et al., 1992; Arnaud et al., 2004; Lopez-Pedrosa et al., 2007).

[23] Technical Dossier/Section 7.8.3

Toxicological information on NAA and NAG[24]

On request of the EFSA GMO Panel the applicant provided toxicological information on NAA and NAG including the full reports of the available toxicological studies. A summary of these studies has been published by Harper et al. (2009), Delaney et al. (2008) and Karaman et al. (2009).

According to information from the scientific literature, injection of NAA into the brains of rats caused seizures, altered EEG recordings and abnormal behaviour (Akimitsu et al., 2000; Kitada et al., 2000). However, there are no reports of adverse effects after oral intake of NAA.

(a) Acute toxicity testing[25]

In acute oral toxicity studies using male and female Sprague-Dawley rats there were no indications of adverse effects after administration of N-acetyl-L-aspartate and N-acetyl-L-glutamic acid at doses of 2000 mg/kg bw. When N-acetyl-L-aspartate was tested at a dose of 5000 mg/kg bw four of five female animals in the test group died and the surviving female as well as all male rats showed signs of toxicity including ataxia, abnormal gait, breathing noise or diarrhoea.

(b) Repeated-dose toxicity testing[26]

Subacute (28-day) feeding study with NAA

N-acetyl-L-aspartic acid was administered in the diet to groups of 10 male and 10 female Sprague-Dawley rats for 28 days. The study was conducted under Good Laboratory Practice (GLP) compliance and in accordance with OECD guideline 407 apart from the selection of dose levels. During the first 14 days the animals received target doses of 10, 100 or 1000 mg/kg bw and during the remaining period the target doses were 100, 500 or 1000 mg/kg bw. The control group received a standard rodent diet. There were no deaths during the treatment period. Clinical signs as well as effects identified in ophthalmological examinations were not related to the test material. Although no statistically significant differences were observed at the end of the treatment period, males and females of the high-dose group showed a tendency of lower body weight gain as well as a slightly lower absolute feed intake and feed efficiency in relation to the control group. A functional observation battery (FOB) and motor activity evaluations did not reveal relevant differences between groups. Urine analyses showed differences in ketone concentrations for male rats, which can be considered as normal variation. Haematology and clinical-chemistry analyses showed several statistically significant differences in the high-dose group compared with the control group, i.e. a lower eosinophil count, lower levels of plasma creatinine and blood urea nitrogen and a higher plasma glucose level in males as well as a lower neutrophil count in females. These differences are not considered toxicologically relevant and most likely represent incidental findings. Other statistically significant differences, each observed in only one of the lower dose groups and in one sex, are also regarded as incidental. Organ weight determinations as well as macroscopic and microscopic examination of organs and tissues at necropsy did not reveal relevant differences in findings between the test and control groups. Therefore in this study no adverse effects were observed up to the highest dose administered. The no observed adverse effect level (NOAEL) in this study was the highest dose administered which corresponds to an actual average dose of 852.3 mg/kg bw for males and 890.1 mg/kg bw for females.

Subacute (28-day) feeding study with NAG[27]

Applying a similar study design N-acetyl-L-glutamate was administered to Sprague-Dawley rats at target doses of 0, 100, 500 or 1000 mg/kg bw/day for 28 days. An additional group received L-glutamate at a target dose of 1000 mg/kg bw/day for comparison. All animals survived during the

[24] Additional information April 2008, March 2010 and April 2010
[25] Additional information March 2010
[26] Additional information March 2010
[27] Additional information March 2010

treatment period and there were no relevant differences between groups regarding body weight development, feed intake and feed efficiency. A functional observation battery (FOB) and motor activity evaluations as well as eye examinations did not reveal relevant findings. In haematology and clinical-chemistry examinations, the only statistically significant differences in relation to the control group were a higher calcium level in females of the mid-dose group, which is considered incidental, as well as higher white blood cell and absolute lymphocyte counts in male rats of the high-dose group. Similar changes in blood cell counts were also observed in male rats receiving L-glutamate. In the absence of any other relevant findings in the other examinations (urinalysis, organ weight determinations, macroscopic and microscopic examinations), these differences, which were not observed in females, are most likely not attributable to administration of NAA (and L-glutamate, respectively). The NOAEL in this study was the highest dose administered, which corresponds to an actual average dose of N-acetyl-L-glutamate of 914.2 mg/kg bw/day for males and 1006.6 mg/kg bw/day for females.

Subchronic (90-day) feeding study with NAA[28]

The applicant also provided a subchronic (90 days) feeding study with N-acetyl-L-aspartic acid using Sprague-Dawley rats. Groups of 10 male and 10 female animals received diets containing N-acetyl-L-aspartic acid at target doses of 100, 250 and 500 mg/kg bw/day. The control group received a standard rodent diet. An additional group was administered L-aspartic acid at a target dose of 500 mg/kg bw/day. The study was conducted in accordance with OECD guideline 408 and under Good Laboratory Practice (GLP) compliance. All animals survived the treatment period. Clinical observations as well as ophthalmological examinations did not reveal relevant differences between the treatment groups and the control group. A functional observation battery (FOB) and motor activity evaluations also did not show relevant differences. Body weight, body weight gain, feed consumption and feed efficiency were comparable in all groups. Haematology examinations showed statistically significantly higher red blood cell counts in females of the high-dose group in relation to the control group. The difference was small and, in the absence of changes in related parameters, not considered toxicologically relevant. Other statistically significant differences observed at lower dose levels were unrelated to the dose and thus regarded as incidental findings (higher white blood cell counts and lymphocyte counts in males; prolonged activated partial thromboplastin time (APTT) in females). Clinical-chemistry examinations showed lower blood urea nitrogen levels in males of the high-dose group, which is not considered as an indication of toxicity. This also applies to a lower creatinine level in males of the mid-dose group. Urine analyses showed no relevant differences. In organ weight determinations carried out at necropsy, males of the mid-dose group showed a higher relative heart weight (in relation to brain), which was not dose-related and not observed in relation to bodyweight and therefore regarded as incidental. A similar conclusion can be drawn for differences in thymus weights observed in female animals (thymus weight in relation to bodyweight was higher in the mid-dose group, whereas thymus weight in relation to brain weight was a lower in the low-dose group). Females of all dose groups showed lower relative liver weight in relation to bodyweight, which was also not related to the dose level and, in the absence of other findings indicating liver toxicity, probably attributable to a relatively high value of the control group. Macroscopic examinations revealed numerous red areas of the thymus of a number of animals in all groups, in particular in female animals of the group administered L-aspartic acid, which was not further explained by the author of the study report but is not considered treatment-related. Microscopic examinations did not reveal relevant differences between groups except for an increased incidence and severity of hypertrophy of the mucus-secreting cells (acinar cells) in the submandibular salivary gland of male and female rats of the high-dose NAA group (but not in the group administered 500 mg L-aspartic acid /kg bw/day). The cells were enlarged with an increased amount of pale, basophilic cytoplasm but there was no evidence of injury or cytotoxicity, e.g. inflammation, degeneration, necrosis or hyperplasia. This effect was observed at a lower incidence and intensity also in the parotid salivary glands (high-dose males and females) and in the sublingual salivary gland (high-dose males). The

[28] Additional information April 2010, July 2010 and December 2010

effect was not observed at a target dose of 250 mg NAA/kg bw/day. The EFSA GMO Panel concludes that the no observed effect level (NOEL) in this study is the mid dose administered, corresponding to an actual average dose of 229.5 and 253.2 mg NAA/kg bw/day for male and female rats, respectively.

Considering the intake of NAA resulting from consumption of 200 g unprocessed 356043 soybeans per day, which can be considered as a conservative assumption for high consumers in the EU (see below the assessment of the EFSA DATEX Unit), the increase in NAA intake, when compared with consumption of 200 g conventional soybeans, would be 1.9 mg/kg bw/day (114 mg/day for a person with 60 kg bw). This is more than 100 fold lower than the NOEL in the 90-day rat feeding study.

Reproductive and developmental toxicity study with NAA[29]

N-acetyl-L-aspartic acid was also tested in a two-generation reproduction toxicity study in rats, which was carried out according to OECD guideline 416 and under GLP compliance. The test material was administered in the diet at target doses of 100, 250 and 500 mg/kg bw/day. The control group received a standard rodent diet (carrier control group), and an additional group was administered L-aspartic acid at a target dose of 500 mg/kg bw/day (comparative control group). Groups of 25 male and 25 female Sprague-Dawley rats (P1 generation) continuously received the test substance in the diet starting 70 day before mating, through mating and continuing until sacrifice. F1 generation rats received the same diet concentrations from weaning until sacrifice or for at least 70 days before mating, through mating and continuing until sacrifice. F2 generation rats received the same diet concentrations from weaning until scheduled sacrifice. Several deaths occurring prior to scheduled sacrifice in the P1, F1 or F2 generation were considered incidental. Regular observations of P1, F1 and F2 animals did not reveal clinically relevant effects, and body weights, body weight changes, feed consumption and feed efficiency were comparable in all groups. Organ weight determinations, macroscopic and microscopic examinations at necropsy did not show relevant differences between groups except for hypertrophy of acinar cells of salivary glands in male and female rats in the F1 generation and male rats from the F2 generation of the high-dose NAA group. Neurohistopathological evaluation provided no evidence that NAA had any effects on brain development. Delivery or litter observations for the P1 or F1 generation females were not affected. There were also no signs of reproductive effects on the P1 or F1 generation males or females or effects on the viability and growth in the F1 or F2 generation offspring. Reproductive parameters evaluated in the F1 and F2 generation rats after weaning were not affected. The EFSA GMO Panel noted a decreased motor activity of male and female animals receiving NAA or L-aspartic acid in one specific subset of the F2 generation (examined on day 22 postpartum). This was not observed in the F1 generation and in older animals (examined on day 61 postpartum) of the F2 generations .Therefore the EFSA GMO Panel considers it unlikely that the observed difference in motor activity is attributable to NAA.

(c) Genotoxicity testing[30]

Tests on induction of gene mutations in bacteria (Ames test) were conducted in accordance with OECD guideline 471. Using the plate incorporation method N-acetyl-L-aspartate and N-acetyl-L-glutamic acid did not induce gene mutations in *Salmonella enterica* var. Typhimurium strains TA98, TA100, TA1535 and TA1537 and *E. coli* strain WP2*uvr*A up to the highest tested concentration of 5000 µg/plate both in the absence and presence of tissue homogenate with metabolic activity (S9-mix).

N-acetyl-L-aspartate and N-acetyl-L-glutamic acid were also tested in the mouse bone marrow micronucleus test. Groups of 10 male and 10 female mice received by gavage N-acetyl-L-aspartate at doses of 500, 1000 or 2000 mg/kg bw as well as N-acetyl-L-glutamic acid at doses of 0 (vehicle control), 333, 1000 or 2000 mg/kg bw. In these tests, which were conducted in accordance with OECD

[29] Additional information, April 2010, July 2010 and July 2010
[30] Additional information April 2010

guideline 474, the test materials did not increase the frequency of micronucleated polychromatic erythrocytes up to the highest dose level tested.

On the basis of this information the EFSA GMO Panel concludes that there is no concern with regard to genotoxicity.

Intake information / Exposure assessment[31]

NAA and NAG are present in conventional foodstuffs and are thus normal constituents of the human diet. In the original application the applicant provided analytical data for a range of foodstuffs, which were selected because they have relatively high concentrations of aspartic acid and glutamic acid. In the study NAA and NAG were determined in yeast extract (ca. 12.6 and 159.8 mg/kg fresh weight (fw), respectively; average values from two samples), chicken bouillon (12.1 and 0.36 mg/kg fw, respectively), whole egg (1.38 and 0.05 mg/kg fw, respectively), ground beef (1.1 and 1.5 mg/kg fw, respectively), ground turkey (4.0 and 0.8 mg/kg fw, respectively) and other products. Additional studies were provided showing that NAA and NAG are also present in other foodstuffs including sardines, apples, oranges, spinach, rice, barley, wheat, walnuts, beer, coffee beans and brewed coffee, tea, cocoa powder and chocolate.

Furthermore, the normal intake of L-aspartic acid and L-glutamic acid resulting from consumption of food protein is 7.3 g aspartic acid plus asparagine and 8.5 g L-glutamic acid per day (Health Council of the Netherlands, 1999) .

On request of the EFSA GMO Panel the applicant provided a dietary exposure assessment for NAA and NAG considering the substitution of conventional soybean by 356043 soybean. Separate studies were carried out using data provided by the US Dietary Exposure Evaluation Model – Food Commodity Intake Database (DEEM - FCID) and the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) Consumption Cluster Diets, respectively. The processed soybean products included in these calculations were defatted flour, protein isolate, soymilk and refined soybean oil. Considering the categorisation of soybean products in the DEEM–FCID and GEMS/Food databases, the applicant has applied specific factors in the assessment, which take into account the impact of processing on the levels of NAA and NAG in the above mentioned processed products.

Using DEEM-FCID, which was based on food consumption surveys conducted in 1994 and 1998, mean and 90th percentile intakes of NAA and NAG were calculated for the US population, including several sub-populations, at baseline (0 %), 45 % and 100 % replacement of materials derived from conventional soybean by materials from soybean 356043. The intake of NAA was estimated to increase from 9.4 µg/kg bw/day at baseline to 16.8 µg/kg bw/day (mean), and from 21.9 to 34.6 µg/kg bw/day (90th percentile) at a 100 % inclusion rate. Regarding a person with a bodyweight of 60 kg, this corresponds to an increase from ca. 570 to 1000 µg/person/day (mean) and from 1310 to 2080 µg/person/day (90th percentile). Thus, the estimated additional intake of NAA at a 100 % inclusion rate considering high consumption is 770 µg/day. For comparison, this amount would also be contained in ca. 290 g ground turkey or 75 g sardines. The intake of NAG was estimated to increase from 2.5 µg/kg bw/day at baseline to 2.7 µg/kg bw/day (mean), and from 5.8 to 6.2 µg/kg bw/day (90th percentile) at a 100 % inclusion rate . For a person with a bodyweight of 60 kg, the latter corresponds to an increase in intake from 348 to 372 µg/person/day. The estimated additional intake of NAG at a 100 % inclusion rate (90th percentile) is thus 24 µg/day, an amount which is also contained in ca. 115 g sardines or 2 g dark chocolate.

An intake estimate concerning infants was also provided. Considering all infants, the intake of NAA was estimated to be increased from 4.3 µg/kg bw/day at baseline to 7.0 µg/kg bw/day (mean), and

[31]Additional information April 2008

from 15.6 to 19.5 µg/kg bw/day (90th percentile) at a 100 % inclusion rate. At a 100 % inclusion rate the NAG intake would thus increase from 13.8 to 14.4 µg/kg bw/day (90th percentile).

The intake assessment based on GEMS/Food Consumption Cluster Diets made use of Food Balance Sheet data compiled by the Food and Agriculture Organisation of the United Nations (FAO). With regard to European countries, the applicant selected the country cluster with the highest predicted dietary exposure for the assessment. The intake of NAA was estimated to increase from 22.8 µg/kg bw/day at baseline to 393.0 µg/kg bw/day at a 100 % inclusion rate, corresponding to an increase from ca. 1.4 to 23.6 mg NAA/day for a 60 kg person. The intake of NAG would increase from 6.6 µg/kg bw/day at baseline to 13.0 µg/kg bw/day, corresponding to an increase from approximately 400 to 780 µg/kg day for a 60 kg person. For the US the intake of NAA was estimated to increase from 26.1 µg/kg bw/day at baseline to 1.2 mg/kg bw/day at a 100 % inclusion rate. The intake of NAG would increase from 8.0 µg/kg bw/day to 28.3 µg/kg bw/day. For an individual with a bodyweight of 60 kg this corresponds to an additional intake of NAA and NAG of ca. 70 and 1.7 mg/day, respectively. Compared with the results of the DEEM/FCID assessment for the US population, the estimated increases in the intakes of NAA and NAG obtained in the GEMS/Food assessment are considerably higher. This can be anticipated considering the differences in the databases and the methodology applied in the assessment.

With regard to the situation in EU countries, the DATEX Unit of EFSA has conducted an additional assessment for high consumers of soybeans assuming a daily consumption of 200 g of unprocessed soybeans (equivalent to 440 g of cooked soybean due to water absorption) for an individual with a bodyweight of 60 kg bw. Analysis of the EFSA Comprehensive Food Consumption Database (EFSA, 2011) has confirmed that 200 g/day is a conservative assumption for unprocessed soybeans. Under this assumption, the intake of NAA would increase from 0.008 mg/kg bw/day (assuming an NAA content in soybeans of 2.52 mg/kg) to 1.9 mg/kg bw/day (assuming an NAA content in soybeans of 580 mg/kg). The intake of NAG would increase from 0.005 mg/kg bw/day (assuming an NAG content in soybeans of 1.53 mg/kg) to 0.04 mg/kg bw/day (assuming an NAG content in soybeans of 11.6 mg/kg). Considering an individual with a bodyweight of 60 kg, the additional intake of NAA and NAG would thus be ca. 114 and 2.1 mg/day, respectively.

On the basis of data from the EFSA Comprehensive Food Consumption Database (EFSA, 2011) it can be anticipated that the daily intake of soybeans by toddlers (12 months - 3 years) and children (3 – 9 years) is lower than that of adults. On a bodyweight basis this may give rise to intake levels, which are lower or slightly higher than those of adults. Therefore the anticipated increases in intake levels of NAA and NAG on a bodyweight basis would be similar to those of adults.

Regarding the intake assessment for infants consuming infant formula, the relevant soybean-derived products to be considered are protein isolate and soybean oil. According to the information provided by the applicant, NAA and NAG were not detected or below the limit of quantification in protein isolate and degummed and refined/bleached/deodorised (RBD) soybean oil (see section 5.1.2). Therefore no increase in the intake of these constituents by infants due to consumption of infant formula containing protein isolate and RBD soybean oil derived from soybean 356043 is expected.

Regarding farm animals the EFSA GMO Panel has estimated the intake of NAA and NAG, which may result from the use of meal derived from soybean 356043 in animal feed. This assessment was based on the assumptions that the diets fed to ruminants and non-ruminants contain high amounts of soybean meal , (i.e. 20% and 15 % of dry mass for dairy cows and beef cattle respectively and 20-30, 20 and 30-40 % of dry mass for growing pigs, laying hens and broilers, respectively) and soy protein (i.e. 30% of soy protein is applied in milk replacer for suckling calves) exclusively derived from soybean 356043. Based on the maximum levels of NAA and NAG in soybean seed (according to the data provided in the application) and assuming that ca. 20 % of the total seed mass is removed by processing, the maximum levels of NAA and NAG in oil extracted meal would amount to approximately 1,300 mg/kg dry weight and 60 mg/kg dry weight, respectively. It was thus estimated that the intake of NAA would vary from 3.7 mg/kg bw/day for beef cattle to 160 mg/kg bw/day for

young broilers (age up to one week). In relation to the estimated intake of NAA, the intake of NAG would be negligible (below 1 mg/kg bw/day for each animal category).

Conclusion

NAA and NAG are normal constituents in the mammalian metabolism. They are also present in conventional foodstuffs and thus consumed as part of a normal diet. The available scientific information indicates that under normal conditions NAA and NAG, like other N-acetylated amino acids, are deacetylated in the intestine to form the corresponding L-amino acids, which are further metabolised in the body. Regarding the exposure assessment the Panel has considered all available data but focused on data from EU countries for soybean consumers. Considering the outcome of a conservative intake assessment (assuming an intake of 200 g unprocessed 356043 soybeans per day instead of unprocessed conventional soybeans), the estimated increase in intake of NAA (114 mg/day) is more than 100 fold lower than the NOEL in the 90-day rat feeding study. Furthermore, in relation to the normal intake of L-aspartic acid and L-glutamic acid resulting from consumption of food protein (see section 5.1.3.3/*Intake information*) the estimated increases in the intake of NAA and NAG are considered low. Considering all the available information, the EFSA GMO Panel is of the opinion that the estimated increases in intake levels of NAA and NAG resulting from replacement of food products derived from conventional soybeans by the respective products derived from soybean 356043 do not raise safety concerns. The same conclusion applies to the use of feed materials derived from this genetically modified soybean.

5.1.3.5. Information on heptadecanoic, heptadecenoic and heptadecadienoic acid

Intake information / Exposure assessment[32]

The levels of the odd chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acid in seeds from soybean 356043 soybean were found to be about two to three times higher than those in its conventional counterpart and outside the ranges for seeds from other commercial non-GM soybean varieties (see section 4.1.2). The contents of heptadecanoic acid, heptadecenoic acid and heptadecadienoic are less than 0.5 %, less than 0.3 %, and about 0.2 %, respectively, of total fatty acids in soybean 356043.

Heptadecanoic, heptadecenoic and heptadecadienoic acid are normal constituents of the human diet. According to the applicant, there are no published studies on the catabolism of heptadecanoic, heptadecenoic and heptadecadienoic acid in mammals. It can be anticipated, however, that these fatty acids are metabolised in a similar way as even chain fatty acids by ß-oxidation generating acetyl-CoA, the entry molecule for the citric acid cycle. The terminal metabolite is expected to be propionyl-CoA (instead of acetyl-CoA), which can be converted to succinyl-CoA, an intermediate of the citric acid cycle. Heptadecanoic and heptadecenoic acid are also found in human tissues, namely heptadecanoic acid in skeletal muscle (Andersson et al., 2002) and subcutaneous adipose tissue (Baylin et al., 2002), and heptadecenoic acid in myocard tissue (Shenolikar, 1980). Both fatty acids are found in human breast milk and erythrocyte membrane lipids (Wendel, 1989). The information on heptadecadienoic acid is more limited.

According to the information provided in the application, the heptadecanoic acid content was 0.54 g/100 g tofu, 0.56 g/100 g butter, 0.29 g/100 g pork, 0.32-0.34 g/100 g beef and 0.3-1.16 g/100 g cooked lamb. The heptadecenoic acid content was 1.09 g/100 g tofu, 0.16-0.2 g/100 g beef, 0.15 g/100 g cheese and 0.13 g/100 g olive oil. Additional information based on a literature search, which was provided on request of the EFSA GMO Panel, showed that heptadecanoic and heptadecenoic acid are present in a wide variety of foodstuffs from plant and animal sources. The applicant has identified this fatty acid in various soy products, shortening, margarine, walnuts as well as several oils (walnut,

[32] Additional information April 2008, December 2010

flaxseed, wheat germ, grapeseed and safflower oil). Heptadecadienoic acid was identified in pecan oil (Senter and Horvat, 1978), cuttlefish oil and beef tallow (Kurata et al., 2005).

On request of the EFSA GMO Panel the applicant provided an exposure assessment for the odd-chain fatty acids, which was based on the consumption of soybean oil, the major product for human consumption. In refined-bleached-deodorized (RBD) 356043 soybean oil the mean levels of heptadecanoic, heptadecenoic and heptadecadienoic acid were 0.343, 0.193, and 0.144 g/100 g, respectively. Using data from the FAOSTAT databases as well as annual production and trade data (1961-2005), the daily consumption of soybean oil was assessed on a per capita basis for 18 European countries, the EU and the USA. The EU average per capita daily soybean oil consumption was estimated to be 10.3 g/day, with the Netherlands showing the highest consumption level (36.1 g/day).

Regarding the potential increase in consumption of the odd-chain fatty acids through replacement of soybean oil with oil derived from soybean 356043, the applicant considered two scenarios compared with the baseline situation, i.e. 45 % and 100 % replacement. For the Netherlands the intake of heptadecanoic acid was estimated to rise from 40 mg/day at baseline to 78 mg/day at a 45 % inclusion rate, and 124 mg/day at a 100 % inclusion rate. For comparison, the additional amount consumed in the worst case situation (84 mg/day) would also be contained in ca. 22 g butter or 43 g pork. In the case of heptadecenoic acid the intake was estimated to increase from 10 mg/day to 37 mg/day at the 45 % inclusion rate, and 70 mg/day at the 100 % inclusion rate. The additional maximum amount consumed (60 mg) would also be contained in 40 g cheese or 33 g beef. Regarding heptadecadienoic acid the intake was estimated to rise from 10 mg/day to 29 mg/day at a 45 % inclusion rate, and 52 mg/day at a 100 % inclusion rate.

Conclusion

The odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acid are normal constituents of plants and animals and have been identified in human tissues. There is no information in the scientific literature indicating that the intake of small amounts of these fatty acids via food or feed causes adverse effects. The EFSA GMO Panel is of the opinion that the estimated increases in intake levels of heptadecanoic, heptadecenoic and heptadecadienoic resulting from replacement of conventional soybean oil with oil from soybean 356043 do not raise safety concerns. The same conclusion applies to the use of feed materials derived from this genetically modified soybean.

5.1.3.6. Toxicological assessment of the whole GM food/feed[33]

The applicant has provided the report on a subchronic (92 days) rat feeding study, which was also published in the scientific literature (Appenzeller et al., 2008). Groups of 12 male and 12 female rats (Crl:CD(SD)) were fed diets containing 20% (w/w) dehulled/defatted toasted meal and 1.5% (w/w) toasted ground hulls derived from soybean 356043 treated or not treated with glyphosate, chlorimuron and thifensulfuron (two test groups). The control group received diets formulated with processed meal and hulls from the conventional counterpart (Jack). Three additional groups were fed diets containing corresponding quantities of the respective feed materials derived from other commercial non-GM soybean varieties (reference groups). In the statistical analysis the data obtained for both test groups were compared separately with the data for the non-GM control group.

Throughout the treatment period there was no mortality except for one male animal in one of the reference groups, and no clinically relevant reactions were noted in the regular observations of the animals. Food consumption was comparable in all groups and there were no relevant differences in food efficiency and body weight development. In ophthalmic examinations as well as quantitative assessments of body functions and motor activity measurements, no statistically significant differences between the groups were detected. In haematology examinations female rats of the test group receiving materials derived from soybean 356043 treated with the target herbicides showed

[33] Technical Dossier/D 7.8.4 and additional information September 2008

statistically significantly higher mean MCV (mean corpuscular volume) and MCH (mean corpuscular haemoglobin) values compared with the control group. In the absence of differences in other red blood cell parameters these relatively small differences in MCV and MCH values are not considered toxicologically relevant. In addition, no differences were observed in male and female animals fed meal and hulls derived from soybean 356043 not treated with the target herbicides. Clinical-chemistry and urine analyses did not reveal significant differences except for a higher mean BUN (blood urea nitrogen) value for male rats of the test group receiving materials derived from soybean 356043 treated with the target herbicides. Since there were no changes in other parameters related to kidney function this difference, which was attributable to one animal in the group, can be considered as an incidental finding. Furthermore, no differences were noted for male and female rats fed materials from soybean 356043 not treated with the target herbicides. Determination of the weights of selected organs and tissues did not reveal statistically significant differences. In macroscopic and microscopic examinations no differences between the test groups and the control group were detected, which are related to administration of the test materials. The EFSA GMO Panel concludes that there are no indications of adverse effects in this subchronic feeding study.

5.1.4. Allergenicity [34]

The strategies used when assessing the potential allergenic risk focus on the characterisation of the source of the recombinant protein(s), the potential of the newly expressed protein(s) to induce sensitisation or to elicit allergic reactions in already sensitised persons and whether the transformation may have altered the allergenic properties of the modified food. A weight-of-evidence approach is recommended, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (CAC, 2003; EFSA, 2011)

5.1.4.1. Assessment of allergenicity of the newly expressed proteins

The gene encoding the *Glycine max*-HRA protein originates from soybean meaning that the source is a common allergenic food. This issue is addressed when assessing the allergenicity of the whole plant since the recipient of the genetic modification is also soybean. The amino acid sequence of the mature form of this protein differs from that of the endogenous acetolactate synthase (ALS) protein in two out of 604 amino acids.

Bacillus licheniformis, the source of the gene encoding the GAT4601 protein, is a common soil bacterium which is not known to cause allergy.

Bioinformatics-supported comparisons of the amino acid sequence of the *Glycine max*-HRA precursor protein and the GAT4601 protein with the sequences of known allergens were performed. These analyses included both an overall search for sequence alignments using the FASTA algorithm and a search for short identical stretches of at least eight contiguous amino acids. No similarity applying a criterion of 35% identity over a window of 80 amino acids was identified and no identical stretches of at least eight contiguous amino acids were detected.

The studies on *in vitro* digestibility of the proteins (see section 5.1.3.2.) showed that most of the proteins were degraded.

Based on this information the EFSA GMO Panel considers that the proteins *Glycine max*-HRA and GAT4601 present in soybean 356043 are unlikely to be allergenic in the intended conditions of use of 356043 soybean.

[34] Technical Dossier/ Section D7.9 and Additional information April 2008 and October 2009

5.1.4.2. Assessment of allergenicity of the whole GM plant [35]

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.

Because the soybean is a recognised allergenic food, the applicant has performed *in vitro* allergenicity studies with extracts of seeds from soybean 356043 and its conventional counterpart (Jack). On the basis of one-dimensional (1-D) IgE immunoblot analysis and ELISA inhibition tests using pooled sera from 5 patients reactive to soybean (both children and adults) and pooled sera from non-atopic individuals as a negative control (number of individuals not given), the applicant concluded that the extracts of seeds from soybean 356043 and its conventional counterpart Jack had very similar protein/allergen profiles and inhibition patterns.

On request of the EFSA GMO Panel the applicant provided additional information. A 2-D immunoblot analysis and a quantitative ELISA analysis for soybean specific IgE were conducted using individual sera from 8 subjects with clinically confirmed allergy to soybeans, 5 negative control sera and one positive control serum. Besides extracts from soybeans 356043 and its conventional counterpart (Jack) extracts from 8 commercial non-GM soybean varieties were analysed in this study. In the 2-D immunoblot analysis no meaningful qualitative and quantitative differences in the IgE binding patterns were detected between extracts of soybean 356043 and its non-GM comparator. In the quantitative ELISA analysis extracts from soybeans 356043, its conventional counterpart and the 8 commercial non-GM soybean varieties had similar IgE binding capacity when tested using the sera from all allergic patients.

Based on the information provided, the EFSA GMO Panel concludes that the overall allergenicity of the whole GM soybean 356043 is unlikely to be different from that of its conventional counterpart and commercial soybean varieties.

5.1.5. Nutritional assessment of GM food/feed [36]

A 42-day feeding study using broiler chickens (Ross x Cobb broilers) was performed according to the ILSI (2003) recommendations. The full report of this study was provided and the results were also published by McNaughton et al. (2007). Groups consisting of 60 male and 60 female animals (12 pens with 5 male and 12 pens with 5 female animals per group (initial body weight ca. 51 g/chick) were fed with diets containing meal from soybean 356043 not treated with herbicides or treated with glyphosate, chlorimuron and thifensulfuron (two test groups). The inclusion rate of soybean meal in the starter, grower and finisher diets was approximately 30%, 26% and 21.5%, respectively. Hulls and oil derived from soybean 356043 (not treated or treated with the target herbicides) were added to all diets at 1% and 0.5%, respectively. The control group received diets formulated with meal and hulls from the conventional counterpart (Jack). Three additional groups were fed diets containing the respective feed materials derived from commercial soybean varieties (reference groups). The diets were adjusted for their contents in protein, specific amino acids and minerals according to NRC (1994). Birds were provided feed and water *ad libitum*. Animal performance on the various diets was evaluated by measuring mortality, weight gain (overall final weight ca. 1910 g/animal), feed consumption, feed conversion ratio (FCR ca. 1.87 g/g bw), organ (kidney, liver) and carcass (breast, thigh, leg, wing, abdominal fat) yields.

There were no statistically significant differences in mortality, weight gain, feed conversion ratio (corrected for mortalities) and carcass yields between the two test groups and the control group, and overall survival was >98%. The only statistically significant difference, a higher mean liver weight only in males fed meal from soybean 356043 treated with the target herbicides, was not considered biologically relevant since the difference was small, not observed in the group fed meal from untreated

[35] Additional information October 2009
[36] Technical Dossier D.7.10.1 and D7.10.2

soybean 356043, and the values were within the ranges determined for three additional groups fed meal from other commercial soybean varieties. Thus, the broiler feeding study shows that diets formulated with meal, hulls and oil derived from soybean 356043 are as nutritious as diets formulated with the respective materials derived from the conventional counterpart and non-GM references soybean varieties included in the study.

5.1.6. Post-market monitoring of GM food/feed[37]

An evaluation of the risk assessment concluded that no data have emerged to indicate that soybean 356043 is any less safe and nutritious than its conventional counterpart and commercial soybean varieties. Therefore, and in line with the EFSA GMO Panel guidance document (EFSA, 2011), the Panel is of the opinion that post-market monitoring of the food/feed derived from soybean 356043 is not necessary.

5.2. Conclusion

No toxicity of the GAT4601 and the *Glycine max*-HRA proteins was observed in acute oral toxicity studies and repeated-dose (28 days) feeding studies using mice. The studies on *in vitro* digestibility of the proteins showed that most of the proteins were degraded. In bioinformatics studies the proteins showed no homology to known toxic proteins and allergens.

The odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acid are normal constituents of plants and animals and have also been identified in human tissues. There is no information indicating that the intake of small amounts of these fatty acids via food or feed causes adverse effects. The EFSA GMO Panel is of the opinion that the estimated increases in intake levels of heptadecanoic, heptadecenoic and heptadecadienoic resulting from replacement of conventional soybean oil with oil from soybean 356043 do not raise safety concerns.

NAA and NAG are normal constituents in the mammalian metabolism. They are also present in conventional foodstuffs and thus consumed as part of a normal diet. The available scientific information indicates that under normal conditions NAA and NAG, like other N-acetylated amino acids, are deacetylated in the intestine to form the corresponding L-amino acids, which are further metabolised in the body. Regarding the exposure assessment the Panel has considered all available data but focused on data from EU countries for soybean consumers. Considering the outcome of a conservative intake assessment, the estimated increase in intake of NAA is more than 100 fold lower than the NOEL in the 90-day rat feeding study with NAA. Furthermore, in relation to the normal intake of L-aspartic acid and L-glutamic acid resulting from consumption of food protein, the estimated increases in the intake of NAA and NAG are considered low. Considering all the available information, the EFSA GMO Panel is of the opinion that the estimated increases in intake levels of NAA and NAG resulting from replacement of food products derived from conventional soybeans by the respective products derived from soybean 356043 do not raise safety concerns. The same conclusion applies to the use of feed materials derived from this genetically modified soybean.

Furthermore, a subchronic 92- day feeding study in rats using diets including meal and hulls derived from soybean 356043 provided no indications of adverse effects. Testing of extracts from soybeans 356043 with sera from patients allergic to soybean showed that the overall allergenicity of the whole plant had not been changed. A 42-day feeding study using broiler chickens demonstrated that soybean 356043 is nutritionally equivalent to its conventional counterpart and commercial non-GM soybean varieties included in this study. Therefore, the EFSA GMO Panel is of the opinion that soybean 356043 is as safe as its conventional counterpart with respect to potential effects on human and animal health in the context of its intended uses.

[37] Technical Dossier D.7.11

6. Environmental risk assessment and monitoring plan

6.1. Environmental risk assessment

The scope of application EFSA-GMO-UK-2007-43 is for food and feed uses, import and processing and does not include cultivation. Considering the intended uses of soybean 356043, the environmental risk assessment is concerned with the exposure through manure and faeces from animals feeding seed produced by soybean 356043 and with the accidental release into the environment of viable seeds of soybean (e.g. during transportation and processing).

As the scope of the present application excludes cultivation, environmental concerns related to the use of glyphosate and ALS-inhibiting herbicides on soybean 356043 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 (herbicides) 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[38]

Cultivated soybean *(Glycine max* (L.) Merr.) is a species belonging to the subgenus *Soja* of the genus *Glycine*. The species originated from eastern Asia and is a highly domesticated crop (Lu, 2005). The major worldwide soybean producers are the United States (US), Brazil, Argentina, China, North Korea and South Korea. In European Union (EU), soybean is mainly cultivated in Austria, Italy, France, Hungary and Romania (Dorokhov et al., 2004). (EUROSTAT[39]). 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 North and South America during the years 2005 (6 locations) and 2005-2006 (6 locations). These field trials did not show changes in plant characteristics that indicate altered fitness and invasiveness of GM soybean 356043 compared to its conventional counterpart, except in the presence of glyphosate and ALS-inhibiting 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 soybean 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 glyphosate and ALS-inhibiting herbicides tolerance traits introduced by the genetic modification result in increased invasiveness of any crop species, except when glyphosate and ALS-inhibiting herbicides are applied. Thus, the accidental release of GM soybean 356043 seeds would not result in 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 glyphosate and ALS-inhibiting herbicides.

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 356043, it can be considered that soybean 356043 has no altered survival, multiplication or dissemination characteristics. Therefore, the EFSA GMO Panel is of the opinion that the likelihood of unintended environment effects of the soybean 356043 in Europe will not be different to that of conventional soybean varieties.

[38] Technical dossier / section D9.1
[39] http://epp.eurostat.ec.europa.eu/portal/page/portal/agriculture/data/database

6.1.2. Potential for gene transfer[40]

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 356043 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 exposure of bacterial communities to the recombinant genes in soybean 356043 must be seen in the context of the natural occurrence and level of exposure to alternative sources of similar genes to which bacterial communities are continually exposed. The inserted DNA includes the glyphosate detoxifying glyphosate acetyltransferase (*gat*) gene which originates from the soil bacterium *Bacillus licheniformis*. It has been subjected to an extensive previous gene shuffling process (Castle et al., 2004) for structural optimization. Sequence similarities between the recombinant gene and its natural counterparts in bacteria may however still be sufficient to increase the likelihood of HR. However, such a hypothesised horizontal gene transfer event would only replace an existing gene. Theoretically, a recombined *gat* gene may cause altered enzyme activities towards glyphosate and other amino acids of a recipient bacterium. However, it is unlikely that this enzyme would increase the fitness of the recipient in context of its natural habitat.

Soybean 356043 also contains the recombinant *Glycine max*-HRA gene encoding for an acetolactate synthase (ALS). This gene, however, is a sequence modified version of the als gene of *Glycine max* which decreases the likelihood for homologous recombination with bacterial genes. The unlikely case of a successful transfer of the *Glycine max*-HRA gene from soybean 356043 to gut or other environmental bacteria would not confer a new trait, because genes encoding for acetolactate synthase (ALS) are expected to be widespread in bacteria and fungi that produce the amino acids leucine, isoleucine and valine.

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 been detected in studies that have exposed bacteria to high

[40] Technical dossier / section D9.2

concentrations of GM plant DNA (EFSA, 2009). In the extreme unlikely event of such a horizontal gene transfer, expression of the recombinant genes in bacteria would be limited by the plant-specific promoters of soybean 356043.

In the context of its intended uses as food and feed, there is no direct exposure of bacteria to the herbicidal compound glyphosate and ALS-inhibiting herbicides. The selective advantage of glyphosate and ALS-inhibiting herbicides resistance in bacteria is therefore predicted to be limited. The hypothetical rare acquisition of the genes encoding for GAT4601 as well as of the *Glycine max*-HRA from soybean 356043 is therefore not considered to confer an advantage that would allow bacteria to enhance their viability or to alter their habitat range.

The EFSA GMO Panel concludes that the recombinant DNA in soybean 356043 does not represent an environmental risk in relation to its potential for horizontal transfer to bacteria in the context of its intended uses.

(b) Plant to plant gene transfer

Considering the intended uses of soybean 356043 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, 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 and the occasional soybean plant 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 356043 seeds (while most soybean 356043 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.

In conclusion, since soybean 356043 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 356043 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 (glyphosate and ALS-inhibiting herbicides with no target organisms) and the intended uses of soybean 356043, which exclude cultivation, this was not considered an issue by the EFSA GMO Panel.

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

Due to the intended uses of soybean 356043, 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 356043, which exclude cultivation and due to the low level of exposure to the environment, potential interactions 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[41]

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 content of the monitoring plan provided by the applicant (EFSA, 2006b). The potential exposure to the environment of soybean 356043 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).

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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 356043 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-UK-2007-43 is for food and feed uses, import and processing of soybean 356043 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 356043 and with the accidental release into the environment of viable seeds of soybean 356043 (e.g. during transportation and processing).

If case of accidental release into the environment of viable seeds of soybean 356043 (e.g. during transport and processing), there are no indications of an increased likelihood of establishment and spread of feral soybean 356043 plants, except in the presence of glyphosate and ALS-inhibiting 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. In the context of its intended uses, the theoretically possible transfer of the recombinant genes from soybean 356043 to gut or other environmental bacteria has not been identified to be a risk due to the lack of any selective advantage. 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 356043.

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,b).

The EFSA GMO Panel also recommends that appropriate management systems should be in place to restrict seeds of soybean 356043 entering cultivation as this would require specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2000.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The EFSA GMO Panel was requested to carry out a scientific risk assessment of the soybean 356043 for food and feed uses, import and processing.

Appropriate molecular and bioinformatic analyses of the 356043 soybean insert and its flanking genomic regions have been undertaken. The expression of the genes introduced 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 356043 soybean is sufficient for the safety assessment. The GMO panel considers this to be an adequate analysis and the molecular characterisation does not indicate a safety concern.

No differences were identified between 356043 soybean and its conventional counterpart, except for the newly expressed proteins, for higher levels of the acetylated amino acids N-acetylaspartate (NAA) and N-acetylglutamate (NAG), and the odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acid in seed from 356043 soybean. The levels of these acetylated amino acids and odd chain fatty acids fall outside the natural ranges observed for other commercial non-GM soybean varieties. The overall level of NAA and NAG (taken together) in soybean 356043 was found to be less than 0.15 % of the total amino acids. The total level of odd chain fatty acids amounts to less than 1% of total fatty acids. No statistically significant differences in total amino acid contents in seed were observed between the 356043 soybean and its conventional counterpart. Levels of major fatty acids in 356043 soybean seed were found to be comparable to those observed in the conventional counterpart.

No toxicity of the GAT4601 and the *Glycine max*-HRA proteins was observed in acute oral toxicity studies and repeated-dose (28 days) feeding studies using mice. The Panel is of the opinion that acute toxicity testing of the newly expressed proteins is of little additional value for the risk assessment of the repeated human and animal consumption of food and feed derived from GM plants.The studies on *in vitro* digestibility of the proteins showed that most of the proteins were degraded. In bioinformatics studies the proteins showed no homology to known toxic proteins and allergens.

The odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acid are normal constituents of plants and animals and have also been identified in human tissues. There is no information indicating that the intake of small amounts of these fatty acids via food or feed causes adverse effects. The EFSA GMO Panel is of the opinion that the estimated increases in intake levels of heptadecanoic, heptadecenoic and heptadecadienoic resulting from replacement of conventional soybean oil with oil from soybean 356043 do not raise safety concerns.

NAA and NAG are normal constituents in the mammalian metabolism. They are also present in conventional foodstuffs and thus consumed as part of a normal diet. The available scientific information indicates that under normal conditions NAA and NAG, like other N-acetylated amino acids, are deacetylated in the intestine to form the corresponding L-amino acids, which are further metabolised in the body. Regarding the exposure assessment the Panel has considered all available data but focused on data from EU countries for soybean consumers. Considering the outcome of a conservative intake assessment, the estimated increase in intake of NAA is more than 100 fold lower than the NOEL in the 90-day rat feeding study with NAA. Furthermore, in relation to the normal intake of L-aspartic acid and L-glutamic acid resulting from consumption of food protein, the estimated increases in the intake of NAA and NAG are considered low. Considering all the available information, the EFSA GMO Panel is of the opinion that the estimated increases in intake levels of NAA and NAG resulting from replacement of food products derived from conventional soybeans by the respective products derived from soybean 356043 do not raise safety concerns. The same conclusion applies to the use of feed materials derived from this genetically modified soybean.

Furthermore, a subchronic 92 day feeding study in rats using diets including meal and hulls derived from soybean 356043 provided no indications of adverse effects. Testing of extracts from soybeans 356043 with sera from patients allergic to soybean showed that the overall allergenicity of the whole plant had not been changed. A 42-day feeding study using broiler chickens demonstrated that soybean 356043 is nutritionally equivalent to its conventional counterpart and commercial non-GM soybean varieties included in this study. Therefore, the EFSA GMO Panel is of the opinion that soybean 356043 is as safe as its conventional counterpart with respect to potential effects on human and animal health in the context of its intended uses.

Considering the intended uses of soybean 356043, 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 356043 (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 glyphosate and ALS-inhibiting 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. In the context of its intended uses, the theoretically possible transfer of the recombinant genes from soybean 356043 to gut or other environmental bacteria has not been identified to be a risk due to the lack of any selective advantage. 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 356043. The EFSA GMO Panel is aware that, due to physical characteristics of soybean seed and methods of transportation, accidental spillage cannot be excluded. Therefore, the EFSA GMO Panel recommends that, within general surveillance, appropriate management systems are introduced to actively monitor the occurrence of feral soybean plants in areas where soybean spillage and plant establishment are likely to occur.

In conclusion, the EFSA GMO Panel considers that information available for soybean 356043 addresses the outstanding questions raised by the Member States and that the soybean 356043, 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 MS, dated 11 April 2007, concerning a request for placing on the market of 356043 in accordance with Regulation (EC) No 1829/2003.

2. Acknowledgement letter, dated 17 April 2007(Ref. SR/KL/shv(2007)2084365), from EFSA to the Competent Authority of the MS.

3. Letter from EFSA to applicant, dated 06 August 2007 (Ref.SR/KL/AC/shv(2007)2299681), requesting additional information under completeness check

4. Letter from applicant to EFSA, dated 11 September 2007, providing additional information under completeness check.

5. Letter from EFSA to applicant, dated 28 September 2007, delivering the 'Statement of Validity' for application EFSA-GMO-UK-2007-43 (Ref.SR/KL/shv(2007)2402780), Soybean 356043 submitted by Pioneer under Regulation (EC) No 1829/2003.

6. Letter from EFSA to applicant, dated 20 December 2007 (Ref.SR/KL/shv(2007)2589484), requesting additional information and stopping the clock.

7. Letter from applicant to EFSA, dated 12 February 2008, providing additional information

8. Letter from EFSA to applicant, dated 27 February 2008 (Ref.SR/KL/shv(2008)2720431), requesting additional information and maintaining the clock stopped.

9. Letter from applicant to EFSA, dated 15 April 2008, providing additional information.

10. Letter from EFSA to applicant, dated 22 July 2008 (Ref. PB/KL/md(2008)3185806), requesting additional information and maintaining the clock stopped.

11. Letter from applicant to EFSA, dated 8 September 2008, providing additional information.

12. Letter from EFSA to applicant, dated 8 September 2008 (Ref.PB/ZD/shv(2008)3279118), requesting additional information and maintaining the clock stopped.

13. Letter from applicant to EFSA, dated 15 October 2008, providing additional information

14. Letter from applicant to EFSA, dated 6 November 2008, providing the timeline for submission of response.

15. Letter from applicant to EFSA, dated 27 April 2009, providing the timeline for submission of response.

16. Letter from applicant to EFSA, dated 6 October 2009, providing additional information

17. Letter from EFSA to applicant, dated 8 January 2010 (Ref.PB/KL/AC/lg(2009)4547393), requesting additional information and maintaining the clock stopped.

18. Letter from EFSA to applicant, dated 14 January 2010 (Ref. PB/KL/AC/lg(2010)4567551), requesting additional information and maintaining the clock stopped

19. Letter from applicant to EFSA, dated 19 February 2010, providing the timeline for submission of response.

20. Letter from applicant to EFSA, dated 5 March 2010, providing additional information

21. Letter from EFSA to applicant, dated 28 May 2010 (Ref.PB/KL/AC/mt(2010)4888857), requesting additional information and maintaining the clock stopped

22. Letter from applicant to EFSA, dated 12 July 2010, providing additional information

23. Letter from EFSA to applicant, dated 20 October 2010 (Ref.PB/KL/AC/mt(2010)5257028), requesting additional information and maintaining the clock stopped

24. Letter from applicant to EFSA, dated 7 December 2010, providing additional information

25. Letter from EFSA to applicant, dated 3 February 2011 (Ref. PB/KL/AC/mt(2011)5513291), re-starting the clock

26. Letter from applicant to EFSA, dated 11 March 2011, submitting spontaneously information

27. Letter from EFSA to applicant, dated 18 March 2011 (Ref. PB/KL/EvH/shv(2011)5631533), confirming receipt of information

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