SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 17 February 2017

doi: 10.2903/j.efsa.2017.4738

Scientific opinion on an application by Dow AgroSciences LLC (EFSA-GMO-NL-2012-106) for the placing on the market of genetically modified herbicide-tolerant soybean DAS-44406-6 for food and feed uses, import and processing under Regulation (EC) No 1829/2003

EFSA Panel on Genetically Modified Organisms (GMO),
Hanspeter Naegeli, Andrew Nicholas Birch, Josep Casacuberta, Adinda De Schrijver, Mikołaj Antoni Gralak, Huw Jones, Barbara Manachini, Antoine Messéan, Elsa Ebbesen Nielsen, Fabien Nogué, Christophe Robaglia, Nils Rostoks, Jeremy Sweet, Christoph Tebbe, Francesco Visioli, Jean-Michel Wal, Fernando Álvarez, Michele Ardizzone, Yi Liu, Franco Maria Neri and Matthew Ramon

Abstract

Soybean DAS-44406-6 expresses 5-enolpyruvyl-shikimate-3-phosphate synthase (2mEPSPS), conferring tolerance to glyphosate-based herbicides, aryloxyalkanoate dioxygenase (AAD-12), conferring tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D) and other related phenoxy herbicides, and phosphinothricin acetyl transferase (PAT), conferring tolerance to glufosinate ammonium-based herbicides. The molecular characterisation data and bioinformatics analyses did not identify issues requiring assessment for food/feed safety. The agronomic and phenotypic characteristics revealed no relevant differences between soybean DAS-44406-6 and its conventional counterpart, except for pod count, seed count and yield. The compositional analysis identified no differences requiring further assessment, except for an increase (up to 31%) in lectin activity in soybean DAS-44406-6. Such increase is unlikely to raise additional concerns for food/feed safety and nutrition of soybean DAS-44406-6 as compared to its conventional counterpart and non-GM reference varieties. There were no concerns regarding the potential toxicity and allergenicity of the three newly expressed proteins, and no evidence that the genetic modification might significantly change the overall allergenicity of soybean DAS-44406-6. Soybean DAS-44406-6 is as nutritious as its conventional counterpart and the non-GM soybean reference varieties tested. There are no indications of an increased likelihood of establishment and spread of occasional feral soybean DAS-44406-6 plants, unless exposed to the intended herbicides. The likelihood of environmental effects from the accidental release of viable seeds from soybean DAS-44406-6 into the environment is therefore very low. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of soybean DAS-44406-6. In conclusion, the GMO Panel considers that the information available for soybean DAS-44406-6 addresses the scientific comments raised by Member States and that soybean DAS-44406-6, as described in this application, is as safe as its conventional counterpart and non-GM soybean reference varieties with respect to potential effects on human and animal health and the environment in the context of the scope of this application.

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Keywords: soybean (*Glycine max* cv. Maverick), DAS-44406-6, herbicide tolerance, 2mEPSPS, AAD-12, PAT, Regulation 1829/2003

Requestor: Competent Authority of the Netherlands

Question number: EFSA-Q-2012-00368

Correspondence: GMO@efsa.europa.eu

Panel members: Andrew Nicholas Birch, Josep Casacuberta, Adinda De Schrijver, Mikołaj Antoni Gralak, Philippe Guerche, Huw Jones, Barbara Manachini, Antoine Messéan, Hanspeter Naegeli, Elsa Ebbesen Nielsen, Fabien Nogué, Christophe Robaglia, Nils Rostoks, Jeremy Sweet, Christoph Tebbe, Francesco Visioli and Jean-Michel Wal.

Competing interests: In line with EFSA's policy on declarations of interest, Panel member Guerche Philippe did not participate in the development and adoption of this scientific output.

Acknowledgements: The Panel wishes to thank the following for the support provided to this scientific output: the members of the Working group on Molecular Characterisation, Food and Feed Safety Assessment and Environmental Risk Assessment for the preparatory work on this scientific opinion.

Suggested citation: EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Birch AN, Casacuberta J, De Schrijver A, Gralak MA, Jones H, Manachini B, Messéan A, Nielsen EE, Nogué F, Robaglia C, Rostoks N, Sweet J, Tebbe C, Visioli F, Wal J-M, Álvarez F, Ardizzone M, Liu Y, Neri FM and Ramon M, 2017. Scientific opinion on an application by Dow AgroSciences LLC (EFSA-GMO-NL-2012-106) for the placing on the market of genetically modified herbicide-tolerant soybean DAS-44406-6 for food and feed uses, import and processing under Regulation (EC) No 1829/2003. EFSA Journal 2017;15(3):4738, 33 pp. doi:10.2903/j.efsa.2017.4738

ISSN: 1831-4732

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Summary

Following the submission of an application (EFSA-GMO-NL-2012-106) under Regulation (EC) No 1829/2003 from Dow AgroSciences LLC, the Panel on Genetically Modified Organisms of the European Food Safety Authority (GMO Panel) was asked to deliver a scientific opinion on the safety of genetically modified (GM) herbicide-tolerant soybean (*Glycine max* cv. Maverick) DAS-44406-6 (Unique Identifier DAS-444Ø6-6). The scope of application EFSA-GMO-NL-2012-106 is for import, processing, and food and feed uses of soybean DAS-44406-6 within the European Union (EU), but it excludes cultivation in the EU.

The GMO Panel evaluated soybean DAS-44406-6 with reference to the scope and appropriate principles described in its guidelines for the risk assessment of GM plants. The evaluation addressed the following components of the risk assessment: the molecular characterisation of the inserted DNA and analysis of the expression of the corresponding proteins; the comparative analyses of compositional, agronomic and phenotypic characteristics; the safety of the newly expressed proteins and the whole food/feed with respect to potential toxicity, allergenicity and nutritional characteristics; and the environmental risk assessment (ERA) and the post-market environmental monitoring (PMEM) plan.

Soybean DAS-44406-6 was developed by *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*)-mediated transformation of cotyledonary nodes derived from germinated soybean (*G. max*) cv. Maverick seeds. It expresses the AAD-12, 2mEPSPS and PAT proteins, which confer tolerance to 2,4-D, glyphosate-based and glufosinate ammonium-containing herbicides. The molecular characterisation data established that soybean DAS-44406-6 contains a single insert consisting of the 2mepsps, aad-12 and pat expression cassettes. No other parts of the plasmid used for transformation were detected in soybean DAS-44406-6. Bioinformatic analyses did not indicate significant similarities to toxins and allergens, and genetic stability was demonstrated. The levels of the newly expressed proteins present in soybean DAS-44406-6 were obtained and reported adequately.

Based on the agronomic and phenotypic characteristics of soybean DAS-44406-6 tested under field conditions, none of the differences identified between soybean DAS-44406-6 and its conventional counterpart required further assessment, except for pod count, seed count and yield. Additionally, no relevant differences were observed between DAS-44406-6 and its conventional counterpart with regard to seed germination when tested under controlled conditions. No differences in composition requiring further assessment for food/feed safety were found between soybean DAS-44406-6 and its conventional counterpart, except for a higher lectin activity (up to 31%) in soybean DAS-44406-6.

The increase in lectin activity is unlikely to raise additional concerns for food/feed safety and nutrition for soybean DAS-44406-6 as compared to its conventional counterpart and the non-GM commercial varieties. The safety assessment identified no concerns regarding the potential toxicity and allergenicity of the newly expressed 2mEPSPS, AAD-12 and PAT proteins in soybean DAS-44406-6 and found no evidence that the genetic modification might significantly change the overall allergenicity of soybean DAS-44406-6. The GMO Panel concludes that soybean DAS-44406-6 is as safe and as nutritious as its conventional counterpart and the non-GM soybean reference varieties. The GMO Panel considers that post-market monitoring of food/feed derived from soybean DAS-44406-6 is not necessary, given the absence of safety concerns identified.

Considering the scope of this application, the ERA is concerned with the accidental release into the environment of viable soybean DAS-44406-6 seeds (i.e. during transport and/or processing), and with the exposure of bacteria to recombinant DNA in the gastrointestinal tract of animals fed GM material and those present in environments exposed to their faecal material (manure and faeces).

In the case of accidental release into the environment of viable seeds of soybean DAS-44406-6, there are no indications of an increased likelihood of establishment and spread of occasional feral soybean DAS-44406-6 plants, unless these plants are exposed to the intended herbicides. The GMO Panel is of the opinion that this will not result in different environmental impacts compared to conventional soybean. Considering the scope of the application EFSA-GMO-NL-2012-106, interactions with the biotic and abiotic environment are not considered to be relevant issues. There are no indications of an increased likelihood of establishment and spread of feral soybean DAS-44406-6 plants in the case of accidental release into the environment of viable GM soybean seeds. Bioinformatic analysis of the inserted DNA identified a theoretical possibility of a facilitated double homologous recombination between genes from DAS-44406-6 and bacteria which may occur in soil. The GMO Panel did not identify a concern in relation to the theoretically possible horizontal gene transfer to bacteria owing to the lack of a selective advantage. Therefore, the GMO Panel concludes that soybean DAS-44406-6 would not raise safety concerns in the event of accidental release of viable GM soybean

seeds into the environment. The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of soybean DAS-44406-6 and the GMO Panel guidelines on the PMEM of GM plants.

In delivering its scientific opinion, the GMO Panel took into account application EFSA-GMO-NL-2012-106, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications. In conclusion, the GMO Panel considers that the information available for soybean DAS-44406-6 addresses the scientific comments raised by Member States and that soybean DAS-44406-6, as described in this application, is as safe and as nutritious as its conventional counterpart and non-GM soybean reference varieties with respect to potential effects on human and animal health and the environment in the context of the scope of this application.

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1. Introduction

Soybean DAS-44406-6 was developed to confer tolerance to glyphosate-based herbicides, 2,4-dichlorophenoxyacetic acid (2,4-D) and glufosinate ammonium-based herbicides. Tolerance to glyphosate-based herbicides is provided by the expression of 5-enolpyruvyl-shikimate-3-phosphate synthase (2mEPSPS) from maize, while tolerance to 2,4-D and other related phenoxy herbicides is achieved by the expression of aryloxyalkanoate dioxygenase (AAD-12) from *Delftia acidovorans*. Tolerance to glufosinate ammonium-based herbicides is provided by the expression of phosphinothricin acetyl transferase (PAT) from *Streptomyces viridochromogenes*.[1]

The assessment of potential consumer health risks resulting from 2,4-D residues and its metabolites in soybean DAS-44406-6 is outside the remit of the GMO Panel and needs to be performed upon request of an applicant in the framework of Regulation (EC) No 396/2005.

1.1. Background

On 16 February 2012, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands an application (Reference EFSA-GMO-NL-2012-106) for authorisation of GM soybean DAS-44406-6 (Unique Identifier DAS-4440Ø6-6), submitted by Dow AgroSciences LLC within the framework of Regulation (EC) No 1829/2003 on GM food and feed.[2]

After receiving the application EFSA-GMO-NL-2012-106, and in accordance with Articles 5(2)(b) and 17(2)(b) of Regulation (EC) No 1829/2003, EFSA informed the Member States and the European Commission (EC), and made the summary of the application publicly available on the EFSA website.[3] 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 12 December 2012, 13 February 2013 and 20 March 2013, EFSA received additional information requested under completeness check (on 28 March 2012, 15 January 2013 and 6 March 2013 respectively). On 15 April 2013, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the EC, and consulted nominated risk assessment bodies of Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC[4] following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Member States had 3 months after the date of receipt of the valid application (until 15 July 2013) to make their opinion known.

On 22 July 2013, 6 November 2013, 10 April 2014, 19 June 2014, 10 February 2015, 19 February 2015, 23 April 2015, 17 July 2015, 2 October 2015, 21 January 2016, 16 February 2016, 26 April 2016 [EURL-JRC], 17 May 2016, 26 May 2016, 10 August 2016 and 29 September 2016, the GMO Panel requested additional information from the applicant. The applicant provided the requested information on 8 November 2013, 17 December 2013, 4 August 2014, 2 December 2014, 2 March 2015, 11 May 2015, 22 June 2015, 31 July 2015, 23 November 2015, 18 February 2016, 10 March 2016, 13 May 2016, 18 May 2016, 25 May 2016 [EURL-JRC], 13 June 2016, 1 September 2016 and 26 October 2016. The applicant also spontaneously provided additional information on 13 May 2013, 17 December 2013, 2 December 2014, 11 May 2015, 23 November 2015, 22 December 2015, 18 February 2016, 10 March 2016, 31 March 2016 [EURL sequence info] and 13 May 2016.

In the frame of contract OC/EFSA/UNIT/GMO/2013/01, CFT/EFSA/AMU/2011/01 and OC/EFSA/UNIT/GMO/2014/01, the contractors performed preparatory work and delivered reports on the information provided and the methods applied by the applicant in performing bioinformatic analyses, statistical analyses and toxicological studies, respectively.

In giving its scientific opinion on soybean DAS-44406-6 to the European Commission (EC), 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 6 months from the acknowledgement of the valid application. As additional information was requested by the GMO Panel, the time limit of 6 months was extended accordingly, in line with Articles 6(1), 6(2), 18(1) and 18(2) of Regulation (EC) No 1829/2003.

[1] Dossier: Part II - Section A2.
[2] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.
[3] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2012-00368
[4] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 12.3.2001, p. 1–38.

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).

1.2. Terms of Reference as provided by the requestor

The GMO Panel was requested to carry out a scientific assessment of soybean DAS-44406-6 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 GMO Panel was not requested to give an opinion on information required under Annex II to the Cartagena Protocol. Furthermore, the GMO Panel did not consider proposals for labelling and methods of detection (including sampling and the identification of the specific transformation event in the food/feed and/or food/feed produced from it), which are matters related to risk management.

2. Data and methodologies

2.1. Data

In delivering its scientific opinion, the GMO Panel took into account application EFSA-GMO-NL-2012-106, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications.

2.2. Methodologies

The GMO Panel carried out an evaluation of the scientific risk assessment of soybean DAS-44406-6 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. The GMO Panel took into account the appropriate principles described in its guidelines for the risk assessment of genetically modified (GM) plants and derived food and feed (EFSA GMO Panel, 2011a), the environmental risk assessment (ERA) of GM plants (EFSA GMO Panel, 2010c) and on the post-market environmental monitoring (PMEM) of GM plants (EFSA GMO Panel, 2011b).

The comments raised by Member States are addressed in Annex G of the EFSA overall opinion[3] and were taken into consideration during the evaluation of risk assessment.

3. Assessment

3.1. Molecular characterisation

3.1.1. Evaluation of relevant scientific data

3.1.1.1. Transformation process and vector constructs[1]

Soybean DAS-44406-6 was developed by *A. tumefaciens* (also known as *Rhizobium radiobacter*)-mediated transformation of cotyledonary nodes derived from germinated soybean (*G. max*) cv. Maverick seeds with plasmid vector pDAB8264. Glufosinate ammonium tolerance conferred by the PAT protein was used as a marker during the selection of transformants.

The plasmid pDAB8264 used for the transformation contained three expression cassettes between the right and left borders of the T-DNA: 2m*epsps*, *aad-12* and *pat*.

The 2m*epsps* expression cassette contains the following genetic elements: a promoter and a 5'-untranslated region (UTR) from the *Arabidopsis thaliana* histone H4A748 gene; TPotp C, a chloroplast transit (targeting) peptide derived from the maize and sunflower RuBisCO small subunits; the maize 2m*epsps* gene with two amino acid changes at positions 102 (threonine to isoleucine) and 106 (proline to serine), and the 3'-UTR of the *A. thaliana* histone H4A748 gene, including a transcription terminator. The RB7-MAR matrix attachment region from *Nicotiana tabacum* was positioned next to the 2m*epsps* expression cassette, to facilitate its expression.

The *aad-12* expression cassette contains the following genetic elements: the constitutive *A. thaliana* polyubiquitin UBQ10 promoter, 5'-UTR and intron; a codon-optimized version of the *aad-12* gene from *Delftia acidovorans*; and the 3'-UTR from the ORF23 of *A. tumefaciens* pTi15955 (AtuORF23), which includes a transcription terminator.

The *pat* expression cassette contains the following genetic elements: the promoter and 5'-UTR from *Cassava vein mosaic virus* (CsVMV); a codon-optimized version of the *pat* gene from *S. viridochromogenes*; and the 3'-UTR from the ORF1 of *A. tumefaciens* pTi15955 (AtuORF1), which includes a transcription terminator.

The vector backbone contained elements necessary for the maintenance of the plasmid in bacteria.

3.1.1.2. Transgene constructs in the genetically modified plant[1]

Molecular characterisation of soybean DAS-44406-6 was performed by Southern analysis, polymerase chain reaction (PCR) and DNA sequence analysis, in order to determine insert copy number, size and organisation of the inserted sequences and to confirm the absence of plasmid backbone sequences. The approach used was acceptable both in terms of coverage and sensitivity.

Southern analyses indicated that soybean DAS-44406-6 contains a single insert, which consists of a single copy of the T-DNA in the same configuration as in the pDAB8264 vector. The insert and copy number were confirmed by multiple restriction enzyme/probe combinations covering the T-DNA region and flanking regions. PCR analyses confirmed the results obtained by the Southern analyses. The absence of vector backbone sequences was demonstrated by Southern analysis using backbone-specific overlapping probes.

The nucleotide sequence of the entire insert of soybean DAS-44406-6 together with 1,494 bp of the 5' and 1,885 bp of the 3' flanking regions were determined. The insert of 10,280 bp is identical to the T-DNA of pDAB8264, except for the deletion of the 5' terminal end and the truncation of the 3' terminal end of the T-DNA, and the insertion of 3 bp. A comparison of the flanking regions with the pre-insertion locus indicated that 4,383 bp of the parental genomic sequence, containing fragments of putative retro-transposable element, were deleted at the insertion locus. Approximately 42% of the soybean genome is occupied by retrotransposon-related sequences. Most of these sequences are remnants of ancient retrotransposons and are no longer active. In addition, most of the retrotransposon-related individual sequences do not fulfil a specific role in the genome. Therefore, this deletion does not raise a particular concern. The possible interruption of known endogenous soybean genes by the insertion in event DAS-44406-6 was evaluated by bioinformatic analyses of the pre-insertion locus and of the genomic sequences flanking the insert. The results of these analyses did not reveal the interruption of any known endogenous gene in the soybean DAS-44406-6.[5]

The results of segregation (see below) and bioinformatic analyses established that the insert is located in the nuclear genome.

Updated bioinformatic analyses of the amino acid sequences of the newly expressed 2mEPSPS, AAD-12 and PAT proteins revealed no significant similarities to known toxins and allergens.[5] In addition, updated bioinformatic analyses of the newly created open reading frames (ORFs) within the insert or spanning the junctions between the insert and genomic DNA did not indicate significant similarities to toxins and allergens.[5]

In order to assess the possibility for horizontal gene transfer by homologous recombination (HR), the applicant performed a sequence identity analysis of the regions of bacterial origin in soybean DAS-44406-6. Two elements were identified with sufficient length and identity to support HR (EFSA, 2015), namely the 525 bp fragment of *A. tumefaciens* Ti plasmid pTi15955 containing the 3'-UTR of ORF23 and the 703 bp fragment from the same plasmid containing the 3'-UTR of ORF1. Double HR with these sequences and the Ti plasmid of *A. tumefaciens* is a possible scenario to consider.

The likelihood and potential consequences of plant-to-bacteria gene transfer are described in Section 3.4.1.1.

3.1.1.3. Protein characterisation and equivalence

Soybean DAS-44406-6 expresses three new proteins AAD-12, 2mEPSPS and PAT.

Given the technical restraints in producing large enough quantities for safety testing from plants, these proteins were recombinantly produced in *Pseudomonas fluorescens or Escherichia coli*. Prior to safety studies, a set of biochemical methods was employed to demonstrate the equivalence between

[5] Additional information received on 22/12/2015.

soybean and microbe-derived proteins. Purified proteins from these two sources were characterised and compared in terms of their physicochemical, structural and functional properties.

AAD-12 characterisation and equivalence[6]

SDS-PAGE and Western blot analysis showed that plant and microbe-derived AAD-12 proteins had the expected molecular weight of ~ 32 kDa and were comparably immunoreactive to AAD-12 protein-specific antibodies. In addition, glycosylation detection analysis demonstrated that none of the AAD-12 proteins were glycosylated. Amino acid sequence analysis by mass spectrometry methods showed that both proteins matched their expected sequence. These data also showed that the N-terminal methionine of the microbe-derived protein was deleted but that only a fraction of the plant-derived protein was similarly truncated. A fraction of the plant protein was acetylated in alanine 2. Such modifications are common in eukaryotic proteins (e.g. Polevoda and Sherman, 2000). The C-termini of the plant and the microbial-derived protein were identical and fully matched the theoretical AAD-12 sequence. Functional equivalence was demonstrated by a biochemical *in vitro* activity assay which showed that both proteins had comparable activity for the intended herbicide. Microbial-produced AAD-12 protein was also screened for its ability to utilise certain endogenous plant substrates and none of them were metabolised by AAD-12.[7]

2mEPSPS characterisation and equivalence[6]

SDS-PAGE and Western blot analysis showed that plant and microbe-derived 2mEPSPS proteins had the expected molecular weight of ~ 47.5 kDa and were comparably immunoreactive to 2mEPSPS protein-specific antibodies. In addition, glycosylation detection analysis demonstrated that none of the 2mEPSPS proteins were glycosylated. Amino acid sequence analysis by mass spectrometry methods showed that both proteins matched their expected sequence. These data also showed that besides the N-terminal methionine which was truncated, both N- and C- termini were identical for the plant and microbe-derived proteins. Functional equivalence was demonstrated by a biochemical *in vitro* activity assay which showed that both proteins had comparable activity.

PAT characterisation and equivalence[8]

The equivalence between the plant and microbe-derived PAT proteins was demonstrated by SDS-PAGE and Western blot analysis. The results from these analyses showed that both proteins migrated to the expected molecular weight of ~ 20.5 kDa. In addition, Western blot analysis showed that both proteins were comparably immunoreactive to PAT-specific antibodies. Functional equivalence was demonstrated by a biochemical *in vitro* activity assay which showed that both proteins had comparable activity for the intended herbicide.

The protein characterisation data comparing the structural, biochemical and functional properties of plant and microbial derived 2mEPSPS, AAD-12 and PAT proteins indicate that these proteins are equivalent. Therefore, the GMO Panel accepts the use of the 2mEPSPS, AAD-12 and PAT proteins expressed in bacteria for the safety studies.

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

Protein levels of the 2mEPSPS, AAD-12 and PAT were analysed by enzyme-linked immunosorbent assay (ELISA) in material harvested from replicated field trials across 10 locations in the United States during the 2010 growing season. Samples analysed included leaves (V5 and V10-V12), root (R3), forage (R3) and seeds (R8-maturity) both treated and non-treated with glyphosate, 2,4-D, glufosinate or a combination of the three. The mean values, standard deviations and ranges of protein expression levels in seeds and forage (n = 40) of the 2mEPSPS, AAD-12 and PAT proteins are summarised in Table 1.

[6] Dossier: Part II – Section A4.2 (Study ID 101707).
[7] Additional information received on 18/02/2016 (Study ID: 101617).
[8] Study ID: 102098 and additional information received on 13/5/2016.
[9] Dossier: Part II - Section A 2.2.3.

Table 1: Protein expression data for the 2mEPSPS, AAD-12 and PAT proteins in soybean DAS-44406-6 (μg/g dry weight) seeds and forage

	Untreated	Glyphosate treated	2,4-D treated	Glufosinate treated	Glyphosate, 2,4-D and glufosinate treated
Seeds					
2mEPSPS	21.97[(a)] ± 6.28[(b)]	22.80 ± 6.87	22.17 ± 6.95	22.22 ± 7.43	21.86 ± 6.81
	(8.68–35.80)[(c)]	(8.24–46.80)	(8.94–34.90)	(8.52–35.02)	(8.66–39.85)
AAD-12	27.37 ± 9.70	25.77 ± 6.79	27.34 ± 10.35	27.34 ± 10.02	25.83 ± 6.51
	(6.99–45.40)	(10.04–46.60)	(8.03–43.00)	(9.77–47.20)	(12.60–42.00)
PAT	2.12 ± 0.49	2.15 ± 0.39	2.13 ± 0.36	2.11 ± 0.44	2.11 ± 0.38
	(1.36–3.19)	(1.30–3.05)	(1.38–2.82)	(1.21–3.23)	(1.26–3.04)
Forage					
2mEPSPS	357.09 ± 146.12	400.47 ± 140.66	330.02 ± 109.78	321.92 ± 74.69	367.32 ± 125.39
	(182.40–862.22)	(167.21–1150.00)	(189.20–680.15)	(173.46–539.08)	(154.04–1,196.00)
AAD-12	73.47 ± 20.77	76.04 ± 19.36	72.53 ± 22.59	73.75 ± 20.39	70.73 ± 21.88
	(35.00–122.00)	(40.00–121.00)	(37.00–117.50)	(37.00–123.50)	(38.50–118.00)
PAT	6.19 ± 1.79	6.48 ± 1.87	5.90 ± 1.40	6.72 ± 1.67	6.33 ± 1.54
	(3.55–10.45)	(3.65–10.35)	(3.50–9.65)	(2.90–11.20)	(4.25–9.55)

2mEPSPS: 5-enolpyruvyl-shikimate-3-phosphate synthase; AAD-12: aryloxyalkanoate dioxygenase; PAT: phosphinothricin acetyl transferase.
(a): Average.
(b): Standard deviation.
(c): Range.

3.1.1.5. Inheritance and stability of inserted DNA[10]

Genetic stability of the soybean DAS-44406-6 insert was assessed by Southern analysis of genomic DNA from five consecutive generations. The restriction enzyme/probe combinations used were sufficient to conclude that all the plants tested retained the single copy of the insert and flanking regions, which were stably inherited in subsequent generations.

Phenotypic stability was observed by segregation analysis of the glufosinate tolerance trait of soybean DAS-44406-6. The results supported the presence of a single insertion, segregating in a Mendelian fashion.

3.1.2. Conclusion on the molecular characterisation

The molecular characterisation data establish that soybean DAS-44406-6 contains a single insert consisting of one copy of the 2m*epsps*, *aad-12* and *pat* expression cassettes. Bioinformatic analyses of the sequences encoding the newly expressed proteins and other ORFs present within the insert or spanning the junctions between the insert and genomic DNA did not indicate significant similarities to toxins and allergens. The stability of the inserted DNA and of the introduced herbicide tolerance traits was confirmed over several generations. The levels of the 2mEPSPS, AAD-12 and PAT protein were obtained and reported adequately. The protein characterisation data comparing the structural, biochemical and functional properties of plant and microbial derived 2mEPSPS, AAD-12 and PAT proteins indicate that these proteins are equivalent and the microbial derived proteins can be used in the safety studies.

3.2. Comparative analysis

3.2.1. Evaluation of relevant scientific data

3.2.1.1. Choice of comparator and production of materials for the comparative assessment[11]

Application EFSA-GMO-NL-2012-106 presents data on agronomic and phenotypic characteristics, as well as data on forage and seed composition of soybean DAS-44406-6 derived from field trials performed at 11 sites in the United States in 2010 and 2012 (Table 2).

[10] Dossier: Part II - Section A 2.2.4.
[11] Dossier: Part II – Sections A3.1–3.2 and additional information received 13/5/2013, 17/12/2013, 2/12/2014 and 11/5/2015.

Table 2: Overview of comparative assessment studies with soybean DAS-44406-6 provided in application EFSA-GMO-NL-2012-106

Study focus	Study details	Comparators	Commercial reference varieties
Agronomic and phenotypic characteristics; composition	Field trials, USA, 2010 (10 locations) and 2012 (one location)	Maverick	Eleven non-GM varieties
Agronomic and phenotypic characteristics	Seed germination test	Maverick	None

GM: genetically modified.

Field trials for the comparative assessment of soybean DAS-44406-6 were performed in the United States at 10 sites in 2010 and one site in 2012, all located in the major soybean-growing regions of the country.[12] All field trials were managed according to local requirements. At each field trial site, the materials were grown in a randomised complete block design with four replicates. The materials were: DAS-44406-6 with no additional herbicide treatment (DAS-44406-6/untreated); DAS-44406-6 additionally sprayed with 2,4-D (DAS-44406-6/2,4-D); DAS-44406-6 additionally sprayed with glyphosate (DAS-44406-6/glyphosate); DAS-44406-6 additionally sprayed with glufosinate (DAS-44406-6/glufosinate); DAS-44406-6 additionally sprayed with 2,4-D, glyphosate and glufosinate (DAS-44406-6/2,4-D + glyphosate + glufosinate); the comparator Maverick; and three non-GM commercial soybean reference varieties.[13,14]

Soybean DAS-44406-6 was obtained using the non-GM soybean variety Maverick as recipient variety (see Section 3.1.1.1). As documented by the pedigree, the line of soybean DAS-44406-6 used in the field trials was not crossed with other soybean lines. Maverick was used as comparator in the field trials (Table 2), and has the same genetic background as of soybean DAS-44406-6. The GMO Panel considers that this non-GM line is the appropriate conventional counterpart.

Statistical analysis of field trial data

The statistical analysis of the agronomic, phenotypic and compositional data from the 2010/2012 field trials followed the recommendations of the GMO Panel (EFSA GMO Panel, 2010a, 2011a,). This included, for each of the five treatments of soybean DAS-44406-6, the application of a difference test (between the GM soybean and its conventional counterpart) and an equivalence test (between the GM soybean and the set of non-GM soybean reference varieties). The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).[15]

3.2.1.2. Agronomic and phenotypic analysis[16]

Fourteen agronomic and phenotypic characteristics were analysed. Of those, nine[17] could be analysed with the combination of difference and equivalence testing (see Section 3.2.1.1). The outcome of the test of difference and test of equivalence is listed below:

- For DAS-44406-6/untreated, one significantly different endpoint was found: yield (GM: 2,087 g; Maverick: 2,257 g), falling under equivalence category II.
- For DAS-44406-6/2,4-D, no differences were identified.
- For DAS-44406-6/glyphosate, two significantly different endpoints: pod count (GM: 403.9; Maverick: 361.1) and seed count (GM: 881; Maverick: 801.7), both falling under equivalence category II.

[12] Ten sites in 2010 (one in Georgia, Indiana, Michigan. and Missouri, and two each in Iowa, Illinois and Nebraska) and one site in 2012 (Pennsylvania).
[13] In total, nine non-GM soybean reference varieties were used across the eleven field trial sites: Dairyland Seed (DSR) 75213-72, DSR 98860-71, DSR 99914N, DSR 99915, DSR 3510, Porter 75148, Williams 82, Stine 3900-2 and Pioneer 93Y41.
[14] In the 2012 field trial site, DAS-44406-6 was not sprayed with any of the intended herbicides: the design only included DAS-44406-6/untreated, the conventional counterpart and the three reference varieties.
[15] In detail, the four outcomes are: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence).
[16] Additional information received on 2/12/2014.
[17] Early population stand count (in 1 m), days to 50% flowering, days to maturity, plant height (cm), final population stand count (in 1 m), pod count (per 5 plants), seed count (per 5 plants), yield (weight (g) of seeds harvested from each plot), 100 seed weight (g). Pod count and seed count were not measured in the 2012 field trial site.

- For DAS-44406-6/glufosinate, two significantly different endpoints: yield (GM: 2,042 g; Maverick: 2,257 g), falling under equivalence category II, and final stand count (GM: 12.17; Maverick: 13.29), for which the test of equivalence could not be applied because the estimated variability of the reference varieties was too small.
- For DAS-44406-6/2,4-D + glyphosate + glufosinate, one significantly different endpoint: yield (GM: 2,072 g; Maverick: 2,257 g), falling under equivalence category II.

For the remaining five endpoints,[18] which did not fulfil the assumptions for parametric testing, the differences between the GM soybean and the conventional counterpart were tested using a Wilcoxon signed rank test. Statistically significant differences were identified for DAS-44406-6/2,4-D (lodging) and DAS-44406-6/2,4-D + glyphosate + glufosinate (seedling vigour); however, the average values for the GM soybean were within the range of the non-GM commercial reference varieties.

The observed differences in pod count, seed count and yield were considered relevant and are further assessed for their potential environmental impact in Section 3.4.1.1.

Agronomic and phenotypic characteristics tested under controlled conditions[16]

Seed germination of soybean DAS-44406-6 was compared with that of its conventional counterpart under warm and cold conditions in two different studies. In each study, four replicates of 100 seeds for each line, in a complete randomised design, were tested for each of the temperature treatments. The warm treatment consisted of exposure to a constant temperature of 25°C for 5 or 7 days, while the cold treatment consisted of exposure to 10°C for 7 days, followed by additional exposure to 25°C for 7 days. The germination rate of soybean DAS-44606-6 seeds under warm and cold conditions did not differ significantly from that of its conventional counterpart.

3.2.1.3. Compositional analysis

Soybean forage and seeds harvested from the field trials in the United States in 2010 and 2012 were analysed for 87 different constituents (nine from forage[19] and 78 from seeds[20]), including the key constituents recommended by the OECD (OECD, 2001). Considering the data on substrate specificity for AAD-12 (Section 3.1.1.3), the GMO Panel concluded that the spectrum of constituents chosen by the applicant is adequate. Seventeen seed constituents having more than 50% of the observations below the limit of quantification were excluded from the statistical analysis.[21]

Of the remaining 70 constituents, the test of equivalence could not be applied to one forage endpoint neutral detergent fibre (NDF) and two seed endpoints (levels of isoleucine and lysine) because the variation associated with the commercial reference genotypes was too small. Among those three endpoints, only lysine level for DAS-44406-6/2,4-D was significantly different from that of the conventional counterpart (Table 3).

The test of difference and the test of equivalence could be applied together to the remaining 67 endpoints, with the following results:

- For DAS-44406-6/untreated, the test of difference identified statistically significant differences for 27 endpoints (25 endpoints from seeds and two from forage[22]). Of these, 22 endpoints fell

[18] Pod shattering, disease incidence, insect damage, lodging and seedling vigour. Seedling vigour values for the 2012 field trial site could not be provided.

[19] Proximates (crude protein, crude fat, ash, and moisture and carbohydrates by calculation), fibre fractions (acid detergent fibre (ADF) and neutral detergent fibre (NDF)) and minerals (calcium and phosphorus).

[20] Proximates (Protein, fat, ash, moisture and carbohydrates by calculation), fibre fractions (ADF, NDF, total dietary fiber), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, selenium, sodium, zinc), amino acids (alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine), fatty acids (caprylic acid (8:0), capric acid (10:0), lauric acid (12:0), myristic acid (14:0), myristoleic acid (14:1), pentadecanoic acid (15:0), pentadecenoic acid (15:1), palmitic acid (16:0), palmitoleic acid (16:1), heptadecanoic acid (17:0), heptadecenoic acid (17:1), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), γ-linolenic acid (18:3), arachidic acid (20:0), eicosenoic acid (20:1), eicosadienoic acid (20:2), eicosatrienoic acid (20:3), arachidonic acid (20:4), behenic acid (22:0)), vitamins (β-carotene, thiamine HCl, riboflavin, niacin, pantothenic acid, pyridoxine HCl, folic acid, ascorbic acid, α-tocopherol, β-tocopherol, δ-tocopherol, γ-tocopherol, and bioactives (total daidzein equivalent, total genistein equivalent, total glycitein equivalent, lectin (activity), phytic acid, raffinose, stachyose, trypsin inhibitor).

[21] These were: caprylic acid (8:0), capric acid (10:0), lauric acid (12:0), myristic acid (14:0), myristoleic acid (14:1), pentadecanoic acid (15:0), pentadecenoic acid (15:1), palmitoleic acid (16:1), heptadecanoic acid (17:0), heptadecenoic acid (17:1), γ-linolenic acid (18:3), eicosadienoic acid (20:2), eicosatrienoic acid (20:3), arachidonic acid (20:4), sodium, β-carotene and β-tocopherol.

[22] Forage endpoints: ash and moisture. Seed endpoints: arginine, cystine, tryptophan, lectin, total daidzein equivalent, trypsin inhibitor, palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), arachidic acid (20:0), behenic acid (22:0), total dietary fibre, ash, carbohydrates, total fat, calcium, copper, potassium, zinc, niacin, folic acid, α-tocopherol, γ-tocopherol.

under equivalence category I or II and five endpoints under equivalence category III or IV (Table 3).

- For DAS-44406-6/2,4-D, 25 significantly different endpoints (24 in seeds and one in forage) were identified.[23] Of these, 21 endpoints fell under equivalence category I or II and four endpoints under equivalence category III or IV (Table 3).
- For DAS-44406-6/glyphosate, 22 significantly different endpoints (21 from seeds and one from forage) were identified.[24] Of these, 20 endpoints fell under equivalence category I or II and two endpoints under equivalence category III or IV (Table 3).
- For DAS-44406-6/glufosinate, 22 significantly different endpoints (21 from seeds and one from forage) were identified.[25] Of these, 19 seed endpoints fell under equivalence category I or II and three endpoints under equivalence category III or IV (Table 3).
- For DAS-44406-6/2,4-D+glyphosate+glufosinate, 23 significantly different endpoints (22 from seeds and one from forage) were identified.[26] Of these, 21 endpoints fell under equivalence category I or II and two endpoints under equivalence category III or IV (Table 3).

Table 3: Compositional endpoints that are further considered based on the results of the statistical analysis: means (for the conventional counterpart and the GM soybean) and equivalence limits (from the non-GM reference varieties) estimated from field trials in 2010 and 2012

Endpoint	Conventional counterpart	Soybean DAS-44406-6					Equivalence limits from non-GM reference varieties
		Untreated[(a)]	2,4 D[(b)]	Glufosinate[(c)]	Glyphosate[(d)]	2,4 D + glufosinate + glyphosate[(e)]	
Arginine (% AA)	7.473	7.41*	7.444	7.447	7.446	7.446	(7.51, 7.892)
Cystine (% AA)	1.454	1.516*	1.483	1.521*	1.491*	1.516*	(1.263, 1.485)
Glutamic acid (% AA)	17.07	17	16.94*	17.04	16.97	17.01	(17.30, 17.83)
Histidine (% AA)	2.776	2.78	2.808*	2.769	2.777	2.768	(2.598, 2.731)
Lysine (% AA)	6.677	6.744	6.818*	6.71	6.719	6.712	–
Lectin activity (HU/mg protein)	61.26	80.59*	71.72*	73.87*	70.27*	74.12*	(27.37, 71.28)
Total fat (% DM)	18.71	19.21*	18.94	19.1*	18.95	18.91	(17.11, 18.92)
Calcium (mg/g DM)	2.901	3.123*	3.069*	2.935	3.079*	2.952	(1.971, 2.936)

GM: genetically modified; 2,4-D: 2,4-dichlorophenoxyacetic acid; DM: dry matter; % AA: percentage of total amino acids; HU: haemagglutination unit; –, the equivalence test was not applied because of the small variation among the non-GM reference varieties.
For the GM soybean, significantly different entries are marked with an asterisk, while the outcomes of the test of equivalence are differentiated by greyscale backgrounds: white (for equivalence categories I and II and for lysine, for which the test was not applied), light grey (equivalence category III) and dark grey (equivalence category IV).
(a): Sprayed only with conventional herbicides.
(b): Sprayed with 2,4-D.
(c): Sprayed with glufosinate ammonium.
(d): Sprayed with glyphosate ammonium.
(e): Sprayed with 2,4-D, glufosinate ammonium and glyphosate ammonium.

[23] Forage endpoints: moisture. Seed endpoints: alanine, glutamic acid, histidine, leucine, lysine, tryptophan, tyrosine, lectin, trypsin inhibitor, palmitic acid (16:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), arachidic acid (20:0), behenic acid (22:0), ash, carbohydrates, crude protein, moisture, calcium, potassium, zinc, folic acid, α-tocopherol, γ-tocopherol,
[24] Forage endpoints: moisture. Seed endpoints: alanine, cystine, lectin, total daidzein equivalent, total genistein equivalent, trypsin inhibitor, palmitic acid (16:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), arachidic acid (20:0), carbohydrates, moisture, calcium, copper, potassium, zinc, thiamine, folic acid, α-tocopherol, γ-tocopherol.
[25] Forage endpoints: moisture. Seed endpoints: aspartic acid, cystine, lectin, trypsin inhibitor, palmitic acid (16:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), arachidic acid (20:0), behenic acid (22:0), NDF, total dietary fiber, carbohydrates, moisture, total fat, potassium, selenium, thiamine, folic acid, α-tocopherol, γ-tocopherol.
[26] Forage endpoints: moisture. Seed endpoints: aspartic acid, cystine, lectin, raffinose, total daidzein equivalent, trypsin inhibitor, palmitic acid (16:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), behenic acid (22:0), NDF, total dietary fibre, carbohydrates, crude protein, moisture, potassium, zinc, riboflavin, folic acid, α-tocopherol, γ-tocopherol.

No further assessment was deemed necessary for the differences in the five amino acids, total fat and calcium levels (Table 3), owing to the known biochemical roles of the compounds involved and to the small absolute magnitude of the reported changes.

Lectin activity[27] in soybean DAS-44406-6 was significantly different (15–31% higher) than that of the conventional counterpart for all five treatments and fell under equivalence category III for four of the five treatments (Table 3). Because of the known antinutritional properties of soybean lectins, the increase in lectin activity is further assessed for potential impact on food and feed safety in section 3.3.1.

3.2.2. Conclusion on comparative analysis

The increase in lectin activity (up to 31%) observed in soybean DAS-44406-6 with respect to its conventional counterpart is further discussed in Section 3.3.1. The GMO Panel concludes that none of the other differences identified in forage and seed composition between soybean DAS-44406-6 and the conventional counterpart, and none of those identified in the agronomic and phenotypic characteristics, needs further assessment regarding food and feed safety.

Based on the agronomic and phenotypic characteristics of soybean DAS-44406-6 tested, no relevant differences were observed between soybean DAS-44406-6 and its conventional counterpart, except for higher pod count, higher seed count and lower yield for soybean DAS-44406-6. These differences are further assessed for their potential environmental impact in Section 3.4.1.1.

3.3. Food/feed safety assessment

3.3.1. Evaluation of relevant scientific data

3.3.1.1. Effects of processing

Processed products

Soybean DAS-44406-6 will undergo existing production processes used for conventional soybean. No novel production process is envisaged.

Compositional analysis identified an increase in lectin activity (up to 31%) in DAS-44406-6 seeds compared to that of the conventional counterpart. Food/feed processing (e.g. soaking, heating, fermentation) is known to reduce the content and/or activity of soybean endogenous anti-nutrients, including lectins (Liener, 1994; Duranti and Gius, 1997; OECD 2012). The applicant provided data on toasted meal, showing that levels of lectin activity in toasted meal from DAS-44406-6 were strongly reduced compared to those from unprocessed seeds (Table 3).[28]

Newly expressed proteins

a) Effect of temperature on newly expressed proteins

The thermal stability of the bacterial 2mEPSPS protein was evaluated by heating protein solutions for 30 min at 25, 37, 55, 75, and 95°C in a buffer solution, respectively. At temperatures at or above 55°C, the enzymatic activity was reduced by $\geq$ 73% and the protein lost $\geq$ 90% of its immunoreactivity. The molecular mass (approximately 47 kDa) was unchanged.[29] These data are in line with those observed in another study where the temperature dependence of bacterial 2mEPSPS activity was examined at nine different temperatures, ranging from 0 to 69°C, showing a considerable enzyme activity from 40 to 58°C, significantly decreased at temperatures over 58°C.[30]

The thermal stability of the bacterial AAD-12 protein was evaluated by heating protein solutions for 30 min at 50, 70, and 95°C in a phosphate-based buffer solution. At all heating conditions (50–95°C) the enzymatic activity was eliminated and the protein lost more than 99% of its immunoreactivity. The molecular mass (approximately 32 kDa) was unchanged. The temperature dependence of bacterial

[27] The biological activity of soybean lectins was quantified using a haemagglutination assay with animal red blood cells (RBCs) (Liener, 1955). The activity is measured in haemagglutination units (HU): one HU corresponds to the level of test solution (serially diluted) that gives agglutination of 50% of the RBCs. The results of the haemagglutination assay are known to be significantly influenced by the different types/batches of red blood cells (RBC) used: therefore, the results in terms of *absolute* values (HU) cannot be considered reliable enough for the risk assessment. However, the applicant showed that the method is reliable in quantifying *relative* differences between test materials; additional information: 7/3/2016, 13/6/2016 and 1/9/2016.

[28] Dossier: Part II – Section A6.2.

[29] Dossier: Part II – Section A4.2 (Study ID 110461).

[30] Additional information received on 13/5/2013 (Study ID 130470).

AAD-12 protein activity was examined after 6 min at different temperatures, ranging from 1 to 60°C. The temperature dependence of bacterial AAD-12 protein activity was examined after 6 minutes at different temperatures, ranging from 1–60°C, using 2,4-D as a substrate. Considerable activity remained up to 40°C but decreased significantly at the temperatures of 50 and 60°C.[31]

The thermal stability of the bacterial PAT protein was evaluated by heating protein solutions for 30 min at 25, 37, 55, 75, and 95°C in a buffer solution. The molecular mass of the PAT protein (approximately 20 kDa) was unchanged at temperatures ≤ 55°C. At temperatures at or above 55°C, > 99% of the enzymatic activity was lost with no residual activity detected above 75°C. At temperatures at or above 37°C, the soluble PAT protein lost ≥ 91% of its immunoreactivity.[32]

b) Effect of pH on newly expressed proteins

The effect of pH on the *in vitro* activity of the bacterial 2mEPSPS was assessed using a mixed buffer system with pH varying from 3 to 9.5. Activity was observed over a narrow window between pH 5 and 6 with an optimum at pH 5.5.[33]

The effect of pH on the *in vitro* activity of the bacterial AAD-12 was assessed using 2,4-D as a substrate and a mixed buffer system with pH varying from 5.5 to 9.5. Considerable activity after 6 min was observed over a narrow window between pH 6 and 7.5 with an optimum at pH 7.[34]

The effect of pH on the *in vitro* activity of the bacterial PAT was assessed using acetyl CoA and glufosinate as substrates and a mixed buffer system with pH at 3, 8 and 11. The enzyme activity was significantly reduced after 10 min at pH 3 and 11, showing the highest activity at pH 8; the molecular mass (approximately 20 kDa) was unchanged at acidic, neutral and basic pH values.[35]

3.3.1.2. Toxicology

The three proteins (2mEPSPS, AAD-12 and PAT) newly expressed in soybean DAS-444Ø6-6 have been extensively characterised and showed the expected molecular weight, immunoreactivity versus specific antibodies, amino acid sequence and functional activity (Section 3.1.1.3). Newly expressed 2mEPSPS and PAT proteins have been previously assessed by the GMO Panel (e.g. EFSA, 2007, 2009b; EFSA GMO Panel, 2011c, 2013, 2015) and no safety concerns for humans and animals were identified. Updated bioinformatics analysis did not reveal similarities of the 2mEPSPS and PAT proteins to known toxins (Section 3.1.1.2). The GMO Panel is not aware of any new information that would change these conclusions. The GMO Panel concludes that the 2mEPSPS and PAT proteins do not raise safety concerns.

The newly expressed AAD-12 protein is assessed below.

The applicant provided new studies on 2mEPSPS, AAD-12 and PAT proteins from bacterial recombinant systems, which were considered equivalent to the plant-produced proteins (Section 3.1.1.3).

a) *In vitro* degradation studies[36]

The resistance to degradation by pepsin of the bacterial 2mEPSPS, AAD-12 and PAT proteins was investigated in solutions at pH ~ 1.2 in three independent studies. The integrity of the test proteins in samples of the incubation mixture taken at various time points was analysed by SDS-PAGE gel electrophoresis followed by protein staining or by Western blotting. The 2mEPSPS and PAT proteins were degraded by pepsin within 1 min. The AAD-12 protein was degraded by pepsin within 30 s.

b) Acute oral toxicity testing[37]

A bacterial 2mEPSPS protein was administered at the dose of 5,000 mg/kg bw to male and female Crl:CD1(ICR) mice. No adverse effects related to the 2mEPSPS protein were observed.

The bacterial AAD-12 protein was administered by oral gavage at a dose of 2,000 mg/kg bw to male and female Crl:CD1(ICR) mice. No adverse effects related to the AAD-12 protein were observed.

A bacterial PAT protein was administered by oral gavage at a dose of 5,000 mg/kg bw to male and female Crl:CD1 mice. No adverse effects related to the PAT protein were observed.[38]

[31] Dossier: Part II – Section A4.2 (Study ID 101047); additional information: 11/8/2013 (Study ID 120996).
[32] Additional information received on 11/8/2013 (Study ID 120937).
[33] Additional information received on 13/5/2013 (Study ID 130470).
[34] Dossier: Part II – Section A4.2; additional information: 11/8/2013 (Study ID 120996).
[35] Additional information received on 11/8/2013 (Study ID 131205).
[36] Dossier: Part I – Section A5.1 and additional information received on 8/11/2013 and 4/8/2014.
[37] Dossier: Part I – Section A4.2.
[38] Dossier: Part I – Section A4.2. The bacterial recombinant expression system is not specified in the study report.

The GMO 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 consumption of food and feed from GM plants by humans and animals.

c) 28-day repeated dose toxicity study

The applicant provided a 28-day oral repeated dose toxicity study in mice[37] to investigate the potential toxicity of the AAD-12 protein. However, the GMO Panel did not consider the overall study design adequate to identify the potential hazard of the AAD-12 protein, because of the low doses of AAD-12 protein tested (highest target dose level approximately 47 mg/kg bw per day, corresponding to an actual dose of approximately 17 mg/kg bw per day) and the limited number of animals used in treatment groups (five per sex per group) which is not considered sufficient to obtain an adequate statistical power (EFSA GMO Panel, 2011a).

The GMO Panel requested another 28-day oral repeated dose toxicity study in rodents, to support the safety assessment of the AAD-12 protein, with a sufficient number of animals to obtain an adequate statistical power, selecting the doses according to OECD TG 407[39] in order to induce adverse effects at the highest dose, or following a limit test approach if toxicity is not expected. In the second 28-day repeated dose toxicity study using mice,[40] the number of animals per treatment group was in line with the guidance document of the EFSA GMO Panel (2011a), but the highest target dose selected (142 mg/kg bw per day) was too low for an adequate hazard identification, as was also the case in the first 28-day study submitted by the applicant. Therefore the GMO Panel did not consider this study in the risk assessment. The study was withdrawn by the applicant.[41]

The applicant provided a new 28-day oral repeated dose toxicity study in mice,[42] which was conducted in accordance with OECD TG 407 and in compliance with the principles of Good Laboratory Practice (GLP). Groups of singly caged Crl:CD1(ICR) mice (11 per sex per group, approximately 8 weeks old at study start) were administered by gavage the AAD-12 protein (in 0.5% METHOCEL™) at a targeted nominal dose of 1,100 mg/kg bw per day (AAD-12 protein group), the vehicle alone (vehicle control group), or bovine serum albumin (BSA) at a targeted nominal dose of 1,100 mg/kg bw per day (BSA control group).

The AAD-12 protein and BSA protein dosing formulations were prepared daily. Samples of the AAD-12 protein and BSA protein dosing formulations along with vehicle were taken from the first mix, from a mix near the middle, and from a mix towards the end of the study for dose confirmation and homogeneity analyses.

Feed and water were provided *ad libitum*. During the treatment period, the animals were checked daily for mortality and general clinical signs. Detailed clinical observations were conducted on all animals pre-treatment and then weekly. Ophthalmoscopy was carried out before the start and at the end of the treatment period. Body weights were recorded on test days 1, 2, 3, 4, 8, 15, 22 and 29 (terminal body weight) and body weight gains were calculated relative to test day 1. Feed consumption was determined on test days 1–2, 2–3, 3–4, 4–8, 8–15 and 22–29. At the end of the treatment period, blood samples were taken and haematological, coagulation and clinical chemistry analyses were performed. All animals were sacrificed and underwent a detailed necropsy examination with selected organs weighed. Organs and tissues from all animals were subjected to a comprehensive histological examination.

The GMO Panel noted that the AAD-12 protein formulations were prepared daily from the powdered test material stored at approximately 4°C until use. Stability tests on the powdered test material (i.e. the lyophilized AAD-12 protein) were not performed as part of this study. According to the study report,[42] the lyophilized AAD-12 protein was determined to be stable for 81 months under refrigerated storage conditions, as part of previous studies[42]; however, the GMO Panel noted that stability of the AAD-12 protein was not documented in these previous studies. Therefore, the concentration of AAD-12 protein of 33.1% in the powdered test material following storage has not been confirmed. However, as dose confirmation analyses were performed on the dosing formulations both at the start and towards the end of the study, the GMO Panel considered that this is not a major limitation compromising the 28-day study.

[39] OECD (Organisation for Economic Co-operation and Development), Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents. OECD Guidelines for the Testing of Chemicals, Section 4.
[40] Additional information received on 23/11/2015 and 13/5/2016.
[41] Applicant to EFSA letter - 2/3/2016.
[42] Additional information received on 13/5/2016.

The GMO Panel noted that haematology and clinical chemistry analyses were conducted on six mice/gender per group and coagulation (prothrombin time) was conducted on the remaining five animals in each group. The reasoning provided by the applicant was practical limitations in obtaining sufficient quantities of blood from mice for haematology and clinical chemistry, and coagulation examinations in the same animal. However, it is well known that when mice are used as the test animal, additional animals may be needed in each dose group to conduct all required determinations. The GMO Panel also noted that the animals were not fasted prior to necropsy and blood collection, as recommended in OECD TG 407.

The AAD-12 protein group was statistically compared to the BSA control group; the latter was also compared to the vehicle control group in order to assess potential effects of the higher protein intake. For all the continuous parameters a two-way analysis of variance (ANOVA; factors: sex and dose) for the two sexes combined, in order to account for sex–dose interactions, and a one-way ANOVA (factor: dose) separately for each sex were performed. For all parameters, in case a statistically significant dose effect was found with the one- or two-way ANOVA, each individual dose group was compared to the control group using Dunnett's test. For body weight gains, globulin, albumin/globulin ratio, red blood cell (RBC) indices, and differential white blood cell (WBC) counts only descriptive statistics were reported.

The results of the dose confirmation analyses revealed that the average recoveries for AAD-12 and BSA protein in 0.5% METHOCEL™ were 71.7% and 73.2%, respectively, based on nominal dosing suspensions at 110 mg/mL. The average recoveries were therefore within the acceptable experimental variation (70–120%). The GMO Panel noted that based on the measured concentration of AAD-12 protein in the dosing suspension, the actual dose administered was 789 mg/kg bw per day. The results of the homogeneity analyses indicated that the preparations were homogeneously mixed.

The few statistically significant differences between the BSA control and vehicle control groups in the examined parameters were considered by the GMO Panel to be within normal biological variability; therefore, both the vehicle control and the BSA control groups were considered suitable to be used as the control groups for the comparison and evaluation of data from the AAD-12 protein group.

No mortality occurred during the treatment period. The GMO Panel considered that the isolated clinical findings and the few ophthalmic changes observed at the end of the study in the AAD-12 protein group were not treatment-related.

No statistically significant differences in body weight or body weight gain were observed in the AAD-12 protein group compared to the BSA control group.

In comparison with the BSA control group, males of the AAD-12 protein group showed statistically significantly lower feed consumption during specific time periods (days 1–2, 2–3, 15–22, 22–29); these differences were not considered as an adverse treatment-related effect by the GMO Panel as there were no statistically significant differences in body weights and body weight gains in animals of the AAD-12 protein group when compared with the BSA control group.

Haematology analysis showed statistically significantly higher WBC counts in males of the AAD-12 protein group when compared with the BSA control group. This finding was largely due to changes in two individual animals (one with an abscess in the neck region and a granulomatous inflammation around the oesophagus; the other showed chronic inflammation of the mediastinal tissue). The changes which largely resulted from (not significantly) higher values in neutrophil counts[43] were attributed by the applicant to inadvertent trauma associated with repeated oral gavage and not to treatment with the AAD-12 protein. The GMO Panel agrees with the interpretation by the applicant.

The platelet (PLT) count in males treated with the AAD-12 protein was statistically significantly higher compared with the BSA control group. This finding was, according to the applicant,[43] attributed to one animal with inadvertent trauma (abscess in the neck region and a granulomatous inflammation around the oesophagus) related to repeated oral gavaging procedure and not related to the treatment with the AAD-12 protein. After removing this animal from the statistical analysis, the increase in the PLT count showed no statistical significance. The increase was considered by the applicant not to be related to the treatment with the AAD-12 protein. The GMO Panel noted that even after removing this single animal with the highest PLT count ($1{,}836 \times 10^3/\mu L$), the mean PLT count in the male AAD-12 protein group ($1{,}499 \times 10^3/\mu L$, versus $1{,}555 \times 10^3/\mu L$) was still higher compared to both the BSA protein group ($1{,}321 \times 10^3/\mu L$) and the vehicle control group ($1{,}302 \times 10^3/\mu L$). The GMO Panel could not exclude that the increased PLT count was related to the treatment with the AAD-12 protein for the following reasons: (1) the PLT counts for the individual animals in the male AAD-12 protein group

[43] Additional information received on 26/10/2016.

(range 1,386–1,836 × $10^3/\mu L$) were either comparable to or higher than the two highest PLT counts for individual animals in the BSA protein group (1,454 and 1,510 × $10^3/\mu L$) and the vehicle control group (1,398 and 1,511 × $10^3/\mu L$); (2) the SD (87) for the AAD-12 protein group after removing the single animal with the highest PLT count was lower than that for the control groups (BSA protein: 168, vehicle control: 143); and (3) the PLT counts for five of six of the individual animals in the male AAD-12 protein group (range 1,466–1,836 × $10^3/\mu L$) were higher than the historical control range (1,205–1,417 × $10^3/\mu L$) presented in the study report (five studies between 2012 and 2016). However, as the increase was slight and not statistically significant after removing the single animal with the highest PLT count, the GMO Panel considered that this difference was not toxicologically relevant.

In females treated with the AAD-12 protein, the reticulocyte (RET) count was slightly, but statistically significantly lower compared with the BSA control group; the mean value was very close to that of the vehicle control group and within the historical control range. This difference was not considered to be toxicologically relevant by the GMO Panel as there were no differences in related haematological parameters.

There were no other significant differences in haematological and clinical chemistry parameters, or in the prothrombin time. However, the GMO Panel noted that the haematological, clinical chemistry and coagulation examinations were only performed on six or five animals per gender per group and thus, the GMO Panel recommendation to use a higher number of animals (EFSA GMO Panel, 2011a) was not fulfilled for the examination of these parameters.

Organ weight determinations showed no statistically significant differences except for a lower absolute (but not relative) epididymides weight (7.6%) in males treated with the AAD-12 protein compared with the BSA control group. This difference was not considered as toxicologically relevant by the GMO Panel as there was no difference in the relative epididymides weight.

Macroscopic examinations at necropsy revealed no gross pathological findings related to the treatment with the AAD-12 protein. Microscopic examinations of selected organs and tissues identified no treatment-related differences in the incidence and severity of the histopathological findings between the groups.

The GMO Panel noted that the haematological, clinical chemistry and coagulation examinations were only performed on six or five animals per gender per group (in line with OECD TG 407 minimum requirements) and thus, the GMO Panel recommendation to use a higher number of animals to ensure appropriate statistical power (EFSA GMO Panel, 2011a) was not fulfilled for the examination of these parameters. Nevertheless, the GMO Panel concluded that no adverse effects were observed in this study after a 28-day administration of the AAD-12 protein to mice at the dose tested (789 mg/kg bw per day).

Toxicological assessment of components other than newly expressed proteins

No new constituents other than the AAD-12, PAT and 2mEPSPS proteins are expressed in soybean DAS-44406-6.

With the exception of increased lectin activity (up to 31%), no relevant changes in the composition of the GM soybean were detected in the comparative compositional analysis (Table 3).

Lectins are a superfamily of proteins selectively binding carbohydrates and they function as recognition molecules in cell–molecule and cell–cell interactions in a variety of biological systems (Sharon and Lis, 2004; Miyake et al., 2007). Lectins are natural components in plants used for food and feed (Nachbar and Oppenheim, 1980; Peumans and Van Damme, 1996; Vasconcelos and Oliveira, 2004) and are widely distributed among Leguminosae, including soybean (Gupta, 1987). The ingestion of legumes containing high levels of certain lectins may be associated with gastrointestinal effects in humans and animals (Noah et al., 1980; Rodhouse et al., 1990; Bardocz et al., 1995; Grant et al., 1995).

In its toxicological assessment of the increase in lectin activity, the GMO Panel took into account the following:

1) The toxicity of raw soybean lectins is low compared to other commonly consumed legumes (Nasi et al., 2009), consisting of reduced growth performance and transient small intestine hypertrophy in experimental feeding studies (Grant et al., 1995).
2) Current industrial and traditional home processing practices are known to considerably reduce lectin content and/or activity in legumes, including soybean (Liener, 1994; Duranti and Gius, 1997; OECD 2012), and the safe use of soybean depends on such practices (König et al., 2004). However, it cannot be excluded that residual lectin activity is still

present in processed soybean products (Peumans and Van Damme, 1996; Rizzi et al., 2003; Vasconcelos and Oliveira, 2004).

3) The observed increase was considered in the context of the high variability reported for lectin activity and lectin protein content in raw soybean (Becker-Ritt et al., 2004; OECD, 2012; Maria John et al., 2017). Regarding any possible impact of the observed increase on the levels of residual activity in processed products, it can be considered that (measurable) residual activity is also characterised by high variability, as observed across different products (Calderon de la Barca et al., 1991) and between different samples of the same product (Maenz et al., 1999).

Based on these considerations, the GMO Panel is of the opinion that the observed increase (up to 31%) in lectin activity in raw soybean DAS-44406-6 is unlikely to raise additional toxicological concerns for soybean DAS-44406-6 with respect to conventional soybean varieties.

The nutritional impact of the increase in lectin activity in soybean DAS-44406-4 will be discussed in Section 3.3.1.5.

3.3.1.3. Animal studies with the food/feed derived from GM plants

90-day feeding study in rats

The applicant provided a sub-chronic (90-day) feeding study, which was conducted in accordance with OECD TG 408[44] and in compliance with the principles of GLP. Five groups of singly caged Crl:CD (SD) rats (12 per sex per group, approximately 8-week old at study start) were fed balanced diets (formulated in accordance with specifications of the Purina Mills Inc. Certified Rodent LabDiet®5002) containing approximately 20% (weight/weight) dehulled/defatted toasted soybean meal derived from soybean DAS-44406-6 (test diet), from the conventional counterpart Maverick[45] (control diet) or from one of three non-GM commercial soybean varieties (Dairyland 99915, Porter 75148, and Williams 82) (reference diets) for at least 90 days.

Prior to diet preparations, analyses for presence or absence of the three newly expressed proteins AAD-12, PAT, and 2mEPSPS were performed by ELISA in dehulled/defatted toasted meal from soybean DAS-44406-6 and from the conventional counterpart materials. Because the proteins were not detected, PCR was performed to confirm that the event was present only in the dehulled/defatted toasted meal from soybean DAS-44406-6.

Feed and water were provided *ad libitum*. All animals were observed daily for mortality and general clinical signs. Detailed clinical observations (DCO) were conducted on all animals pre-treatment and then weekly. Ophthalmoscopy was carried out before the start and at the end of the treatment period. Body weights and feed consumption were recorded weekly. At the end of the treatment period, blood samples were taken for coagulation; haematology and clinical chemistry and urine samples were taken for urinalysis. At necropsy, all animals underwent a detailed necropsy and selected organs were weighed. A comprehensive histological examination was performed on selected organs and tissues from all animals fed the test and the control diet, as well as on gross lesions from all animals fed the reference diets.

The GMO Panel noted that functional testing was not performed. According to the study report 'No functional testing was performed in this study because previous observations in testing of transgenic soybean products did not reveal any neurotoxicity deficits either in this laboratory (. . .) or in similar studies conducted by others reported in the literature (Appenzeller et al., 2008; Delaney et al., 2008; Chukwudebe et al., 2011).' According to the OECD TG 408 text 'Functional observations conducted towards the end of the study may be omitted when data on functional observations are available from other studies and the daily clinical observations did not reveal any functional deficits'. The GMO Panel interprets the OECD TG 408 text as functional testing can be omitted if data on functional observations are available from other studies with the test material under evaluation. As no treatment-related clinical signs were observed in this study (see below), the GMO Panel considered that the deviation from the OECD TG 408 is not a major deviation compromising the 90-day study.

The GMO Panel noted the following deviations from the recommendations in the EFSA Guidance document on conducting 90-day studies in rodent (EFSA Scientific Committee, 2011): (1) animals were housed individually whereas EFSA recommends that animals of the same sex should be housed in

[44] OECD (Organisation for Economic Co-operation and Development), Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents. OECD Guidelines for the Testing of Chemicals, Section 4.

[45] Dossier: Part II – Section A4.5; additional information received on 2/12/2014.

pairs; (2) only one dose level was used in the study whereas EFSA recommends two dose levels; and (3) a power analysis was not performed.

Statistical analyses were run separately for each sex. The group fed the test diet was independently compared with the group fed the control diet. The groups fed diets containing the reference varieties were used to provide reference ranges. Means and standard deviations were reported for all continuous data and for body weight gains, globulin, albumin/globulin ratio, RBC indices and differential WBC counts. Statistical outliers were excluded from feed consumption analyses. Body weights, feed consumption, organ weights, urine volume, urine specific gravity, clinical chemistry data, coagulation and appropriate haematological data were evaluated by Bartlett's test for equality of variances and, on the basis of these outcomes, a t-test or Wilcoxon rank sum test were performed. DCO incidence data were statistically analysed by a z-test of proportions comparing soybean DAS-44406-6 group versus conventional counterpart group. No correction method for multiple comparisons in the same group was applied.

No mortalities occurred during the treatment period. The GMO Panel considered that the isolated clinical findings and the few ophthalmic changes observed at the end of the study in the test diet group were not treatment-related.

No statistically significant differences in the mean terminal body weight or body weight gain were observed in the test diet group compared to those given the control diet. No statistically significant changes were observed in feed consumption, except for two instances (days 8–15 and days 78–85) of higher values for females fed the test diet compared to female rats given the control diet. The GMO Panel considered that, in the absence of changes in the mean terminal body weight and body weight gain, these differences were not toxicologically relevant.

Statistically significant differences in some haematological and clinical chemistry parameters were observed between animals fed the test diet and the control diet.

The total WBC count was lower in males fed the test diet; the GMO Panel considered that, in the absence of effects in the other immune-related parameters assessed, this difference was not toxicologically relevant.

The RET count was lower in males fed the test diet, and the RBC count, haemoglobin concentration and haematocrit were slightly higher in females fed the test diet. Adverse haematological effects, e.g. anaemia, would have been associated with decreases in RBC parameters and often also with an increase in the RET count. Therefore, the GMO Panel considered that the decrease in the RET count and the increases in RBC parameters were not toxicologically relevant.

The serum aspartate aminotransferase (AST) level was higher, and the cholesterol and triglyceride levels were lower in males fed the test diet. The GMO Panel considered that, in the absence of other effects in the liver, these differences were not toxicologically relevant.

There were no other significant differences in haematological and clinical chemistry parameters, or in the prothrombin time and urinalysis.

Organ weight determinations showed no statistically significant differences between animals fed the test diet and those given the control diet.

Macroscopic examinations at necropsy revealed no gross pathological findings related to the consumption of the test diet. Microscopic examinations of selected organs and tissues identified no treatment-related differences in the incidence and severity of the histopathological findings between the test diet group and the control group.

The GMO Panel noted some limitations in the study (i.e. functional testing was not performed; animals housed individually; only one dose level in the study; and a power analysis was not performed).

The GMO Panel concluded that no adverse effects were observed in this study after a 90-day administration to rats of a diet formulated with 20% dehulled/defatted toasted soybean meal derived from soybean DAS-44406-6.

42-day feeding study in broiler

The applicant provided a 42-day feeding study with a total of 600 (equal numbers of male and female) chickens for fattening (Ross 708, day-old at start).[28] The birds were randomly allocated to five dietary treatment groups with 120 chicks per treatment (12 pens per treatment, six pens for male and six for female, 10 birds per pen). Diets containing soybean DAS-44406-6 (verified by PCR) treated with 2,4-D and glyphosate were compared to diets containing its conventional counterpart or one of three non-GM commercial soybean varieties (Dairyland 99915, Porter 75148 or Williams 82 soybean). Meal was prepared from soybean grains and the formulated isocaloric diets were analysed for proximates,

fibres, isoflavones, minerals, amino acids, anti-nutrients, mycotoxins and pesticides. The chickens were fed starter, grower and finisher diets in mash form containing 39%, 35% and 31% soybean meal, respectively. The nutrient content of the diets was adjusted according to the standards of the National Research Council (NRC, 1994). AAD-12, EPSPS and PAT proteins were detected in grain, but not found in the meals prepared from the grain. Feed and water were provided *ad libitum*.

Chickens were observed twice daily for clinical signs. Body weight and feed intake were measured at day 1, 14, 28 and 42. At day 42, three birds per pen were euthanized and processed for carcass evaluation (yield, dressing percentage, weight of thighs, breast, wings, legs, abdominal fat and whole liver). ANOVA (pen was considered as the experimental unit) was applied to determine statistical differences between groups; pair-wise comparison was made by Dunnett's test.

Mortality was low (1.8%), with no significant difference between groups. No significant treatment × sex interactions was detected for growth parameters. Overall no significant differences between soybean DAS-44406-6 and its conventional counterpart were found for final body weight (ca. 2.4 kg), daily feed intake (ca. 90 g) and feed:gain ratio (1.57). In comparisons made with individual commercial varieties, no relevant differences were found. Carcass parameters did not differ between soybean DAS-44406-6 and the conventional counterpart, except for thigh weight.

The GMO Panel concludes that administration of diets containing up to 39% soybean DAS-44406-6 to broilers, up to 42 days, did not cause adverse effects. Moreover, the measured performance endpoints were similar between groups fed balanced diets containing GM and non-GM soybeans.

3.3.1.4. Allergenicity

The strategies to assess the potential risk of allergenicity focus on the source of the recombinant protein, on the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons and on whether the transformation may have altered the allergenic properties of the modified plant.

Assessment of allergenicity of the newly expressed proteins[46]

A weight-of-evidence approach was followed, taking into account all of the information obtained on the newly expressed protein, as no single piece of information or experimental method yields sufficient evidence to predict allergenicity (EFSA, 2006; Codex Alimentarius 2009; EFSA GMO Panel, 2011a).

The 2m*epsps* gene originates from *Zea mays* L., which is not considered to be a common allergenic food. The *aad-12* gene originates from *D. acidovorans* and the *pat* gene originates from *S. viridochromogenes*, microorganisms which are not considered to be allergenic sources.

Updated bioinformatic analyses[5] of the amino acid sequences of the 2mEPSPS, AAD-12 and PAT proteins, using the criterion of 35% identity in a sliding window of 80 amino acids, revealed no significant similarities to known allergens. In addition, the applicant also performed analyses searching for matches of eight contiguous identical amino acid sequences between the 2mEPSPS, AAD-12 and PAT proteins and known allergens, which confirmed the outcome of the previous bioinformatic analysis.

The study on resistance to degradation of the 2mEPSPS, AAD-12 and the PAT proteins by pepsin has been described in Section 3.3.1.2.a.

The GMO Panel has previously evaluated the safety of the 2mEPSPS and PAT proteins in the context of several applications and no concerns on allergenicity were identified (e.g. EFSA, 2007, 2009b; EFSA GMO Panel, 2011c, 2013, 2015).

There is no information available on the structure or function of the newly expressed AAD-12, 2mEPSPS and PAT proteins that would suggest an adjuvant effect of the individual proteins or their simultaneous presence in soybean DAS 44406-6 resulting in or increasing an eventual IgE response to a bystander protein.

[46] Dossier: Part II – Section A5.1; additional information received on 8/11/2013, 17/12/2013, 4/8/2014 and 22/12/2015.

In the context of the present application, the GMO Panel considers that there are no indications that the newly expressed 2mEPSPS, AAD-12 and PAT proteins, individually or their simultaneous presence, in soybean DAS 44406-6 may be allergenic.

Assessment of allergenicity of the whole GM plant or crop[47]

Soybean is considered to be a common allergenic food[48] (OECD, 2012). Therefore, any potential change in the endogenous allergenicity of the GM plant when compared with that of its appropriate comparator(s) should be assessed in line with the applicable EFSA guidance document (EFSA GMO Panel, 2011a). The applicant performed *in vitro* allergenicity studies and a comparative analysis using extracts from soybean DAS 44406-6, its conventional counterpart and non-GM reference soybean varieties.

Initially, the applicant provided a one-dimensional electrophoresis followed by Western blot and an ELISA inhibition studies using pooled sera from 10 individuals allergic to soybean. In addition, the applicant provided a two-dimensional electrophoresis followed by Western blot or Coomassie blue, and ELISA inhibition studies using single serum from six individuals allergic to soybean. On request from the GMO Panel, the applicant performed a qualitative and quantitative comparative analysis of the two-dimensional Western blots which was carried out by densitometry. According to the applicant, minor variations in spot intensities, which are commonly seen in such studies, were observed between soybean DAS 44406-6 and its conventional counterpart. The GMO Panel did not identify indications of safety concern.

Subsequently, the applicant spontaneously provided a measurement of specific known allergens in soybean by mass spectrometry approaches (i.e. liquid chromatography–mass spectrometry/mass spectrometry (LC–MS/MS)), which has previously been considered as an alternative/complementary acceptable approach for the assessment of endogenous allergenicity in GM plants (EFSA GMO Panel, 2010b; Fernandez et al., 2013). The allergens measured by LC-MS/MS were Gly m 1, Gly m 3, Gly m 4, Gly m 5, Gly m 6, Gly m Bd 28 K, Gly m Bd 30 K and Gly m 8 in soybean seeds. The applicant selected these allergens based on a list of potential soybean allergens described in the OECD consensus document on soybean (2012). The comparative analysis was performed with the combination of difference and equivalence testing recommended by the EFSA GMO Panel (2011a). The outcome of such analysis showed that the genetic modification did not induce relevant changes in the levels of any of the natural endogenous allergens tested.

In the context of this application and considering the information above, the GMO Panel is of the opinion that there is no evidence that the genetic modification might significantly change the overall allergenicity of soybean DAS 44406-6, when compared with that of its conventional counterpart and non-GM commercial reference soybean varieties.

3.3.1.5. Nutritional assessment of GM food/feed

Comparison of the composition of soybean DAS-44406-6 with its conventional counterpart and non-GM reference varieties identified differences in the lectin activity in seeds (up to 31% increase in the GM soybean, Table 3). Lectins are known to be antinutritional factors for humans and animals (Section 3.3.1.2).

The presence of lectins in common plant foods such as Leguminosae is well known (Peumans and Van Damme, 1996; Vasconcelos and Oliveira, 2004). Current industrial and traditional home processing practices are used to considerably reduce lectin content and/or activity in legumes, including soybean (Liener, 1994; Duranti and Gius, 1997; OECD, 2012). It cannot be excluded that residual lectin activity is still present in processed soybean food products (Calderon de la Barca et al., 1991; Peumans and Van Damme, 1996; Rizzi et al., 2003); hence, dietary exposure to functionally active lectins at residual levels in processed products is considered a common event (Nachbar and Oppenheim, 1980; Vasconcelos and Oliveira, 2004).

Soybean oil is the predominant soybean product for human consumption. The oil is obtained by fractionation of soybean seeds and is almost entirely composed of fat, with a negligible non-fat fraction (0.3%) consisting of moisture, insoluble and volatile matter (Gandhi, 2009). Even in the worst-case

[47] Dossier: Section A5.2; additional information received on 2/12/2014 and 22/6/2015.

[48] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

scenario, assuming that the entire non-fat fraction of the oil derived from soybean DAS-44406-6 are lectins, the observed increase in lectin activity in soybean DAS-44406-6 (up to 31%) would not raise concerns compared to oil from non-GM soybeans. Other processing procedures (e.g. soaking, heating and fermentation) are known to considerably reduce active lectin content in soybean (Gupta, 1987, Codex Alimentarius 1989, 2013, 2015; Reddy and Pearson, 1994; Duranti and Gius, 1997; Lajolo and Genovese, 2002; OECD, 2012). Soybean sprouting is also known to be accompanied by a decrease in active lectin content (Rizzi et al., 2003). In the context of this application, it was also demonstrated that toasting is effective in lowering lectin activity of DAS-44406-6 soybean seeds to very low levels.[28] The GMO Panel considers that the additional intake of active lectins from soybean DAS-44406-6 food products (deriving from the 31% increase in the raw material) is likely to be negligible compared to the habitual dietary intake of lectins from non-GM soybean food products.

Soybean meal is the by-product of the extraction of soybean oil and it is the most important protein source used to feed farm animals. In the solvent extraction process, soybeans are cracked, heated, flaked and the oil is extracted by solvent (usually hexane). The obtained by-product is then subjected to treatments that improve its nutritional value by a decrease in the activity of antinutritional factors (e.g. lectins and enzyme inhibitors). Current conditions during the commercial processing of soybean into commodity oil, meal and other products, and on-farm processing conditions (e.g. soaking, roasting, extrusion and micronisation) have consistently shown the ability to reduce the presence and/or activity of lectins and enzyme inhibitors to the extent that they can be consumed by monogastric animals. Quality processes and testing such as urease activity are in place, mainly in commercial production, to assure that many of these commercial products have undergone the proper processing procedures for use in feed applications.[49] Urease activity is highly positively related to lectin and trypsin inhibitor activity (Fasina et al., 2003). The GMO Panel considers that, within the frame of good farming practice described above and considering current processing practices, the observed increase in lectin activity is unlikely to influence animal nutrition.

The GMO Panel concluded that food and feed derived from soybean DAS-44406-6 are expected to have no adverse nutritional impact, as compared to those from its conventional counterpart and commercial non-GM reference varieties.

3.3.1.6. Post-market monitoring of GM food/feed

There was no indication that food/feed products derived from soybean DAS-44406-6 are less safe or nutritious than those derived from its conventional counterpart or the non-GM commercial varieties (Sections 3.2.1.3, 3.3.1.2 and 3.3.1.5). Therefore, in line with EFSA (2006) and EFSA GMO Panel (2011a), the GMO Panel is of the opinion that post-market monitoring of the GM food/feed is unnecessary.

3.3.2. Conclusion on the food/feed safety assessment

The safety assessment identified no concerns regarding the potential toxicity of the 2mEPSPS, AAD-12 and PAT proteins newly expressed in soybean DAS-44406-6, considering their structural and functional properties, the results of bioinformatic analyses and the results of a sub-acute 28-day toxicity study on AAD-12. The GMO Panel did not identify indications of safety concerns regarding allergenicity or adjuvanticity with the 2mEPSPS, AAD-12 and PAT proteins or regarding the overall allergenicity of DAS-44406-6. The observed increase in lectin activity in raw soybean DAS-44406-6 is unlikely to raise additional concerns for food/feed safety and nutrition for the GM soybean as compared to its conventional counterpart and the non-GM commercial varieties. No other changes in soybean DAS-44406-6 composition relevant for food/feed safety and nutrition were identified. Considering current soybean processing, soybean DAS-44406-6 is as safe and nutritious as its conventional counterpart and the non-GM commercial varieties.

3.4. Environmental risk assessment and monitoring plan

3.4.1. Evaluation of relevant scientific data

Considering the scope of application EFSA-GMO-NL-2012-106, which excludes cultivation, the ERA of soybean DAS-44406-6 is mainly concerned with: (1) the exposure of bacteria to recombinant DNA in the gastrointestinal tract of animals fed GM material and bacteria present in environments exposed

[49] Commission Regulation (EU) No 68/2013, on the Catalogue of feed materials http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32013R0068&from=EN

to faecal material (manure and faeces); and (2) the accidental release into the environment of viable seeds of soybean DAS-44406-6 during transportation and/or processing (EFSA GMO Panel, 2010c).

3.4.1.1. Environmental risk assessment

Persistence and invasiveness of the GM plant[50]

Cultivated soybean (*G. max* (L.) Merr.) is a species in the subgenus *Soja* of the genus *Glycine*. The species originated from eastern Asia and is a highly domesticated crop (Lu, 2005). Cultivated soybean seeds rarely display any dormancy characteristics and can grow as volunteers in the year after cultivation only under certain environmental conditions. If volunteers occur, they do not compete well with the succeeding crop, and can easily be controlled mechanically or chemically (OECD, 2000). The presence of volunteers of *G. max* was occasionally reported in some areas of Italy where soybean is intensively cultivated (Celesti-Grapow et al., 2010). However, soybean seeds usually do not survive during the winter owing to herbivory, rotting and germination, or owing to management practices prior to planting the subsequent crop (Owen, 2005). Also, survival of soybean plants outside cultivation areas is limited mainly by a combination of low competitiveness, absence of a dormancy phase, and susceptibility to plant pathogens and cold climatic conditions.

The applicant presented agronomic and phenotypic data on soybean DAS-44406-6 gathered from field trials conducted in soybean growing areas in the United States (Section 3.2.1.2). Soybean DAS-44406-6 (both treated and untreated with the intended herbicides) had lower yields in some comparisons with the conventional counterpart, and glyphosate-treated soybean DAS-44406-6 had higher pod count (+12%) and seed count (+10%) than the conventional counterpart. Considering the observed differences all together, it is unlikely that these will change the fitness (e.g. survival, fecundity, competitiveness) or invasiveness characteristics of soybean DAS-44406-6 plants, given the low survival capacity of soybean.

Further, it is considered unlikely that herbicide tolerance will provide soybean DAS-44406-6 a selective advantage outside cultivation. The application of 2,4-D-, glyphosate- and/or glufosinate-based herbicides is not expected to improve their ability to survive over seasons because of soybean vulnerability to several abiotic and biotic factors. Therefore, it is considered very unlikely that soybean DAS-44406-6 will differ from conventional soybean varieties in its ability to survive until subsequent seasons or to establish feral populations under European environmental conditions.

The GMO Panel is not aware of any scientific report of increased survival capacity, including overwintering, of existing GM soybean varieties (Dorokhov et al., 2004; Owen, 2005; Bagavathiannan and Van Acker, 2008; Lee et al., 2009). Therefore, the GMO Panel is of the opinion that the likelihood of environmental effects of soybean DAS-44406-6 in Europe will not be different from that of conventional soybean varieties.

Effects of gene transfer[51]

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 through vertical gene flow via cross-pollination from feral plants originating from spilled seed.

1) Plant-to-bacteria gene transfer

Genomic plant 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 processing and 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 bacteria in the digestive tract of humans, domesticated animals and other environments exposed to the GM plant or plant material is expected.

Current scientific knowledge of recombination processes in bacteria indicates that horizontal transfer of non-mobile, chromosomally located DNA fragments between unrelated organisms (such as from plants to bacteria) is not likely to occur at detectable frequencies under natural conditions (for further details, see EFSA, 2009a).

A successful horizontal gene transfer would require stable insertion of the recombinant DNA sequences into a bacterial genome and conferring a selective advantage to the transformed host. The only mechanism known to facilitate horizontal transfer of non-mobile, chromosomal DNA fragments to

[50] Dossier: Part II – Section E 3.1.
[51] Dossier: Part II - Sections E3.1 and E3.2 and additional information received on 2/3/2015.

bacterial genomes is HR. This requires the presence of stretches of DNA sequences that are similar in the recombining DNA molecules. In addition to substitutive gene replacement, the insertion of non-homologous DNA sequences is facilitated if their flanking regions share sequence similarity with bacterial sequences in the recipient.

Soybean DAS-44406-6 contains some genetic elements derived from bacterial genome origin. These are (1) the coding sequence of the *aad-12* gene from *D. acidovorans*; (2) the coding sequence of the *pat* gene from *S. viridochromogenes*; (3) two UTRs comprising the transcriptional terminator and polyadenylation site of ORF23 and for ORF1 from plasmid pTI5955 from *A. tumefaciens* (Barker et al., 1983); and (4) an intervening sequence of 228 bp from the Ti plasmid C58 of *A. tumefaciens*, respectively.

Both *aad-12* and *pat* genes were optimized to modify the G+C codon bias to a level more typical for plant expression and thereby the DNA sequences changed to levels (< 90% sequence identity) which would not facilitate HR with native sequences as present in *D. acidovorans* or *S. viridochromogenes*, respectively. Sequence identity sufficient to facilitate HR, however, was found for ORF23 and ORF1 of pTI5955. Furthermore, sequence identity was found for the intervening sequence originating from the *A. tumefaciens* plasmid C58. The similarities between the abovementioned genes and bacterial genes were confirmed by bioinformatic analyses provided by the applicant.

Theoretically, the sequence identities found between the plant-inserted DNA sequences and sequences of the *A. tumefaciens* plasmid C58 could facilitate homologous recombination and with pTI5955 double HR, respectively. Recombination between the intervening sequences with those plasmids present on plasmid C58 would only replace identical or nearly DNA sequences and thus adds no new property. Equally this would be the case for HR between ORF23 and ORF1. However, double HR between both sequences in soybean DAS-44406-6 and pTI5955 in *A. tumefaciens* could result in the insertion of the plant codon-optimized *pat* gene. However, such a recombination and gain of the pat gene would occur at the cost of replacing a large part of the T-DNA region (Barker et al., 1983) and thus producing a plasmid which would have lost its capacity to infect plants.

Agrobacterium tumefaciens or close relatives from the genus *Rhizobium* occur in soil, water and in the plant rhizosphere, but are not typical constituents of the microbial communities as they occur in the main receiving microbial habitat, i.e. the gastrointestinal tract of humans or animals. However, occurrence of the recombinant genes outside the immediate receiving environment (through faecal material) in soils cannot be ruled out (Hart et al., 2009) and is therefore also taken into account for assessing the risks associated with a horizontal gene transfer.

In case that, despite a very low exposure, a recombinant modified pTI5955 plasmid with an inserted *pat* gene, as described above, would be generated in an *A. tumefaciens* strain, it is unlikely that this *pat* gene would provide a selective advantage in the given environment: The gene is optimized for plant expression and regulated by the CsVMV promoter. The expression of such a promoter-gene construct in bacteria is unknown, but generally the expression level of eukaryotic promoters in bacteria is inefficient (Warren et al., 2008). Furthermore, natural variants of *pat* genes have been found in different soil-inhabiting bacteria and thus *pat* genes would not confer a novel trait to soil microbial communities.

In addition to homology-based recombination processes, non-homologous (illegitimate) recombination that does not require the presence of DNA similarity between the recombining DNA molecules is theoretically possible. However, illegitimate recombination events have not been detected in studies that have exposed bacteria to high concentrations of GM plant DNA (EFSA, 2009a). Thus, this process, in comparison with HR, is not considered to contribute significantly to horizontal gene transfer events.

In conclusion, the GMO Panel identified a theoretical possibility of a facilitated double HR between genes from soybean DAS-44406-6 and bacteria which may occur in soil, which however, in addition to a high unlikelihood due to limited exposure and biological restrictions, would not provide a selective advantage to a possible bacterial recipient. Therefore, the GMO Panel concludes that the recombinant DNA in soybean DAS-44406-6 does not represent an environmental risk in relation to its potential for horizontal transfer to bacteria.

2) Plant-to-plant gene transfer

Considering the scope of application EFSA-GMO-NL-2012-106 and the biology of soybean, the potential of occasional feral GM soybean plants originating from seed import spills to transfer recombinant DNA to sexually cross-compatible plants is assessed.

The genus *Glycine* is divided into two distinct subgenera: *Glycine* and *Soja*. The subgenus *Glycine* contains 16 perennial wild species, whereas the cultivated soybean, *G. max*, and its wild and semi-wild annual relatives, *G. soja* and *G. gracilis*, are classified in the subgenus Soja (OECD, 2000). Owing to the low level of genomic similarity among species of the genus *Glycine*, *G. max* can cross with only other members of the *Glycine* subgenus *Soja* under natural conditions (Singh et al., 1987; 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). As *G. soja* and *G. gracilis* are indigenous to China, Taiwan, Korea, Japan, the far-east region of Russia, Australia, the Philippines and the South Pacific, and as they have not been reported in other parts of the world where the cultivated soybean is grown (Dorokhov et al., 2004; Lu, 2005), the plant-to-plant gene transfer from soybean is restricted to cultivated areas and occasional soybean plants resulting from seed spillage in the EU.

Soybean is an annual, almost completely self-pollinating crop with a percentage of cross-pollination usually below 1% (OECD, 2000; Ray et al., 2003; Lu, 2005; Yoshimura et al., 2006; Abud et al., 2007). Soybean pollen dispersal is limited because the anthers mature in the bud and directly pollinate the stigma of the same flower (OECD, 2000).

However, cross-pollination rates as high as 6.3% have been reported for closely spaced plants (Ray et al., 2003), suggesting the potential for some within-crop gene flow in soybean. These results indicate that natural cross-pollination rates can fluctuate significantly among different soybean varieties under particular environmental conditions, such as favourable climate for pollination and an abundance of pollinators (Gumisiriza and Rubaihayo, 1978; Kikuchi et al., 1993; Ahrent and Caviness, 1994; Ray et al., 2003; Lu, 2005).

For plant-to-plant gene transfer to occur, imported soybean DAS-44406-6 seeds need to be processed outside the importing ports, transported into regions of soybean production in Europe, spilled during transportation, germinate and develop into plants in the very close vicinity of soybean fields, and there needs to be an overlap of flowering periods and environmental conditions favouring cross-pollination. It must be noted that most soybean DAS-44406-6 seeds are processed in the countries of production or in ports of importation. The overall likelihood of cross-pollination between feral GM soybean plants and cultivated soybean is therefore extremely low.

In conclusion, even if cross-pollination would occur, the GMO Panel is of the opinion that the likelihood of environmental effects as a consequence of the spread of genes from occasional feral GM soybean plants in Europe will not differ from that of conventional soybean varieties (see previous Section 3.4.1.1).

Interactions of the GM plant with target organisms[52]

Considering the scope of application EFSA-GMO-NL-2012-106, and the absence of target organisms, potential interactions of feral soybean DAS-44406-6 plants arising from seed import spills with target organisms are not considered a relevant issue by the GMO Panel.

Interactions of the GM plant with non-target organisms[53]

Considering the scope of application EFSA-GMO-NL-2012-106, and the low level of exposure to the environment, potential interactions of spilled seeds or occasional feral soybean DAS-44406-6 plants arising from seed import spills with non-target organisms are not considered a relevant issue by the GMO Panel.

Interactions with the abiotic environment and biochemical cycles[54]

Considering the scope of application EFSA-GMO-NL-2012-106, and the low level of exposure to the environment, potential interactions of occasional feral soybean DAS-44406-6 plants arising from seed import spills with the abiotic environment and biogeochemical cycles are not considered a relevant issue by the GMO Panel.

[52] Dossier: Part II - Section E3.3.
[53] Dossier: Part II - Section E3.4.
[54] Dossier: Part II - Section E3.6.

3.4.1.2. Post-market environmental monitoring[55]

The objectives of a PMEM plan, according to Annex VII of Directive 2001/18/EC, are: (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the ERA are correct; and (2) to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment that were not anticipated in the ERA.

Monitoring is related to risk management, and thus a final adoption of the PMEM plan falls outside the mandate of EFSA. However, the GMO Panel gives its opinion on the scientific content of the PMEM plan provided by the applicant (EFSA GMO Panel, 2011b).

The PMEM plan proposed by the applicant for soybean DAS-44406-6 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; and (3) the use of networks of existing surveillance systems (Lecoq et al., 2007; Windels et al., 2008). The applicant proposes to submit a PMEM report on an annual basis, and a final report at the end of the consent period.

The GMO Panel considers the scope of the PMEM plan provided by the applicant is consistent with the scope of soybean DAS-44406-6. As the ERA does not cover cultivation and did not identify potential adverse environmental effects from soybean DAS-44406-6, no case-specific monitoring is necessary. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

3.4.2. Conclusion on the environmental risk assessment and monitoring plan

In the case of accidental release into the environment of viable seeds of soybean DAS-44406-6, there are no indications of an increased likelihood of establishment and spread of occasional feral soybean DAS-44406-6 plants, unless these plants are exposed to the intended herbicides. The GMO Panel is of the opinion that this will not result in different environmental impacts compared to conventional soybean.

Considering the scope of application EFSA-GMO-NL-2012-106, interactions of soybean DAS-44406-6 with the biotic and abiotic environment are not considered to be relevant issues. Bioinformatic analysis of the inserted DNA identified a theoretical possibility of a facilitated double HR between genes from DAS-44406-6 and bacteria which may occur in soil. The GMO Panel did not identify a concern in relation to the theoretically possible horizontal gene transfer to bacteria owing to the lack of a selective advantage.

Therefore, considering the introduced traits, the outcome of the comparative analysis, the routes of exposure and the limited exposure levels, the GMO Panel concludes that soybean DAS-44406-6 would not raise safety concerns in the event of accidental release of viable GM soybean seeds into the environment.

The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of soybean DAS-44406-6 and the GMO Panel guidelines on the PMEM of GM plants (GEFSA GMO Panel, 2011b).

4. Conclusions

The GMO Panel was asked to carry out a scientific assessment of soybean DAS-44406-6 for import, processing, and food and feed uses in accordance with Regulation (EC) No 1829/2003.

The molecular characterisation data and bioinformatics analyses did not identify issues requiring further assessment for food/feed safety.

The agronomic and phenotypic characteristics of soybean DAS-44406-6 tested revealed no relevant differences between soybean DAS-44406-6 and its conventional counterpart, except for pod count, seed count and yield for soybean DAS-44406-6 treated with glyphosate. No differences in composition requiring further assessment for food/feed safety were found between soybean DAS-44406-6 and its conventional counterpart, except for a higher lectin activity (increased up to 31%) in soybean DAS-44406-6. The increase in lectin activity is unlikely to raise additional concerns for food/feed safety and nutrition for soybean DAS-44406-6 as compared to its conventional counterpart and the non-GM commercial varieties. No concerns regarding the potential toxicity or allergenicity of the newly

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expressed 2mEPSPS, PAT and AAD-12 proteins were identified, and no evidence that the genetic modification might significantly change the overall allergenicity of soybean DAS-44406-6 was found. The GMO Panel concludes that soybean DAS-44406-6, assessed in this application, is as safe and as nutritious as its conventional counterpart and the non-GM soybean reference varieties tested. The GMO Panel considers that post-market monitoring of food/feed derived from soybean DAS-44406-6 is not necessary, given the absence of safety concerns identified.

The GMO Panel concluded that there is a very low likelihood of environmental effects resulting from the accidental release of viable seeds from soybean DAS-44406-6 into the environment. Considering the scope of the application with regard to food and feed use, interactions with the biotic and abiotic environment were not considered an issue. Risks associated with an unlikely, but theoretically possible, horizontal gene transfer from soybean DAS-44406-6 to bacteria have not been identified. The scope of the PMEM plan provided by the applicant is in line with the intended uses of soybean DAS-44406-6.

In conclusion, the GMO Panel considers that the information available for soybean DAS-44406-6 addresses the scientific comments raised by the Member States and that soybean DAS-44406-6, as described in this application, is as safe as the conventional counterpart and other non-GM soybean varieties with respect to potential effects on human and animal health and the environment in the context of the scope of this application.

Documentation provided to EFSA

1) Letter from the Competent Authority of the Netherlands received on 16 February 2012 concerning a request for placing on the market of genetically modified soybean DAS-44406-6 submitted by Dow AgroSciences LLC in accordance with Regulation (EC) No 1829/2003 (application reference EFSA-GMO-NL-2012-106).
2) Acknowledgement letter dated 21 March 2012 from EFSA to the Competent Authority of the Netherlands.
3) Letter from EFSA to applicant dated 28 March 2012 requesting additional information under completeness check.
4) Letter from applicant to EFSA received on 7 May 2012 providing a timeline for submission of responses.
5) Letter from applicant to EFSA received on 18 July 2012 extending (1) the timeline for submission of responses.
6) Letter from applicant to EFSA received on 30 July 2012 extending (2) the timeline for submission of responses.
7) Letter from applicant to EFSA received on 21 September 2012 extending (3) the timeline for submission of responses.
8) Letter from applicant to EFSA received on 16 November 2012 extending (4) the timeline for submission of responses.
9) Letter from applicant to EFSA received on 12 December 2012 providing additional information under completeness check.
10) Letter from EFSA to applicant dated 15 January 2013 requesting additional information under completeness check.
11) Letter from applicant to EFSA received on 13 February 2013 providing additional information under completeness check.
12) Letter from EFSA to applicant dated 6 March 2013 requesting additional information under completeness check.
13) Letter from applicant to EFSA received on 20 March 2013 providing additional information under completeness check.
14) Letter from EFSA to applicant dated 15 April 2013 delivering the 'Statement of Validity' of application EFSA-GMO-NL-2012-106 for placing on the market of genetically modified soybean DAS-44406-6 submitted by Dow AgroSciences LLC in accordance with Regulation (EC) No 1829/2003.
15) Letter from applicant to EFSA received on 13 May 2013 providing additional information spontaneously.
16) Letter from EFSA to applicant dated 22 July 2013 requesting additional information and stopping the clock.
17) Letter from EFSA to applicant dated 6 November 2013 requesting additional information and maintaining the clock stopped.

18) Letter from applicant to EFSA received on 8 November 2013 providing additional information.
19) Letter from applicant to EFSA received on 17 December 2013 providing additional information. The package included spontaneous information.
20) Letter from EFSA to applicant dated 10 April 2014 requesting additional information and maintaining the clock stopped.
21) Letter from EFSA to applicant dated 19 June 2014 requesting additional information and maintaining the clock stopped.
22) Letter from applicant to EFSA received on 4 August 2014 providing additional information.
23) Letter from applicant to EFSA received on 2 December 2014 providing additional information. The package included spontaneous information.
24) Letter from EFSA to applicant dated 10 February 2015 requesting additional information and maintaining the clock stopped.
25) Letter from EFSA to applicant dated 19 February 2015 requesting additional information and maintaining the clock stopped.
26) Letter from applicant to EFSA received on 2 March 2015 providing additional information.
27) Letter from EFSA to applicant dated 23 April 2015 requesting additional information and maintaining the clock stopped.
28) Letter from applicant to EFSA received on 11 May 2015 providing additional information. The package included spontaneous information.
29) Letter from applicant to EFSA received on 22 June 2015 providing additional information.
30) Letter from EFSA to applicant dated 17 July 2015 requesting additional information and maintaining the clock stopped.
31) Letter from applicant to EFSA received on 31 July 2015 providing additional information.
32) Letter from EFSA to applicant dated 2 October 2015 requesting additional information and maintaining the clock stopped.
33) Letter from applicant to EFSA received on 23 November 2015 providing additional information. The package included spontaneous information.
34) Letter from applicant to EFSA received on 7 December 2015 providing clarifications.
35) Letter from applicant to EFSA received on 22 December 2015 providing additional information spontaneously.
36) Letter from EFSA to applicant dated 15 January 2016 requesting additional information and maintaining the clock stopped.
37) Letter from EFSA to applicant dated 19 January 2016 withdrawing the EFSA letter of 15 January 2016.
38) Letter from EFSA to applicant dated 21 January 2016 requesting additional information and maintaining the clock stopped.
39) Letter from EFSA to applicant dated 16 February 2016 requesting additional information and maintaining the clock stopped.
40) Letter from applicant to EFSA received on 18 February 2016 providing additional information. The package included spontaneous information.
41) Letter from applicant to EFSA received on 10 March 2016 providing additional information. The package included spontaneous information.
42) Email from applicant to EFSA received on 31 March 2016 providing sequencing info.
43) Letter from EURL-JRC to EFSA dated 22 April 2016 requesting EFSA to stop the clock on behalf of the EURL-JRC.
44) Email from EFSA to applicant dated 26 April 2016 requesting additional information for EURL-JRC and maintaining the clock stopped.
45) Letter from applicant to EFSA received on 13 May 2016 providing additional information. The package included spontaneous information.
46) Letter from EFSA to applicant dated 17 May 2016 requesting additional information and maintaining the clock stopped.
47) Letter from applicant to EFSA received on 18 May 2016 providing additional information.
48) Letter from applicant to EFSA received on 25 May 2016 providing additional information.
49) Letter from EFSA to applicant dated 26 May 2016 requesting additional information and maintaining the clock stopped.
50) Letter from applicant to EFSA received on 13 June 2016 providing additional information.

51) Letter from EFSA to applicant dated 10 August 2016 requesting additional information and maintaining the clock stopped.
52) Letter from applicant to EFSA received on 1 September 2016 providing additional information.
53) Letter from EURL-JRC to EFSA received on 7 September 2016 requesting EFSA to re-start the clock on behalf of EURL-JRC.
54) Letter from EFSA to applicant dated 8 September 2016 re-starting the clock.
55) Letter from EFSA to applicant dated 29 September 2016 requesting additional information and stopping the clock.
56) Letter from applicant to EFSA received on 26 October 2016 providing additional information.
57) Email from EFSA to applicant dated 9 November 2016 re-starting the clock retroactively from 26 October 2016.
58) Letter from EURL-JRC to EFSA dated 15 November 2016 requesting EFSA to stop the clock on behalf of the EURL-JRC.
59) Letter from EFSA to EURL-JRC dated 17 November 2016 providing clarifications on the stop-the-clock request.

References

Abe J, Hasegawa A, Fukushi H, Mikami T, Ohara M and Shimamoto Y, 1999. Introgression between wild and cultivated soybeans of Japan revealed by RFLP analysis for chloroplast DNAs. Economic Botany, 53, 285–291.

Abud S, de Souza PIM, Vianna GR, Leonardecz E, Moreira CT, Faleiro FG, Junior JN, Monteiro PMFO, Rech EL and Aragão FJL, 2007. Gene flow from transgenic to nontransgenic soybean plants in the Cerrado region of Brazil. Genetics and Molecular Research, 6, 445–452.

Ahrent DK and Caviness CE, 1994. Natural cross-pollination of 12 soybean cultivars in Arkansas. Crop Science, 34, 376–378.

Appenzeller LM, Munley SM, Hoban D, Sykes GP, Malley LA and Delaney B, 2008. Subchronic feeding study of herbicide–tolerant soybean DP-356Ø43-5 in Sprague-Dawley rats. Food and Chemical Toxicology, 46, 2201–2213.

Bagavathiannan MV and Van Acker RC, 2008. Crop ferality: implications for novel trait confinement. Agriculture, Ecosystems and Environment, 127, 1–6.

Bardocz S, Grant G, Ewen SWB, Duguid TJ, Brown DS, Englyst K and Pusztai A, 1995. Reversible effect of phytohaemagglutinin on the growth and metabolism of rat gastrointestinal tract. Gut, 37, 353–360.

Barker RF, Idler KB, Thompson DV and Kemp JD, 1983. Nucleotide sequence of the T-DNA region from the *Agrobacterium tumefaciens* octopine Ti plasmid pTi15955. Plant Molecular Biology, 2, 335–350.

Becker-Ritt AB, Mulinari F, Vasconcelos IM and Carlini CR, 2004. Antinutritional and/or toxic factors in soybean (*Glycine max* (L) Merril) seeds: comparison of different cultivars adapted to the southern region of Brazil. Journal of the Science of Food and Agriculture, 84, 263–270.

Calderon de la Barca A, Vázquez-Moreno L and Robles-Burgueno MR, 1991. Active soybean lectin in foods: isolation and quantitation. Food chemistry, 39, 321–327.

Celesti-Grapow L, Pretto F, Carli E and Blasi C (eds.), 2010. *Flora vascolare alloctona e invasiva delle regioni d'Italia*. Casa Editrice Universita La Sapienza, Rome. 208 pp.

Chukwudebe A, Privalle L, Reed A, Wandelt C, Contri D, Dammann M, Groeters S, Kaspers U, Strauss V and van Ravenzwaay B, 2011. Health and nutritional status of Wistar rats following subchronic exposure to CV127 soybeans. Food and Chemical Toxicology, 50, 956–971.

Codex Alimentarius, 1989. Codex general standard for soy protein products. CODEX STAN 175-1989. Available online: www.fao.org/input/download/standards/325/CXS_175e.pdf

Codex Alimentarius, 2009. Foods derived from modern biotechnology. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme. Rome, Italy, p. 85.

Codex Alimentarius, 2013. Regional standard for fermented soybean paste (Asia). CODEX STAN 298R-2009. Available online: www.fao.org/input/download/standards/11251/CXS_298Re.pdf

Codex Alimentarius, 2015. Regional standard for non-fermented soybean products. CODEX STAN 322R-2015. Available online: http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https://workspace.fao.org/sites/codex/Standards/CODEX+STAN+322R-2015/CXS_322Re.pdf

Delaney B, Appenzeller LM, Munley SM, Hoban D, Sykes GP, Malley LA and Sanders C, 2008. Subchronic feeding study of high oleic acid soybeans (Event DP-3Ø5423-1) in Sprague-Dawley rats. Food and Chemical Toxicology, 46, 3808–3817.

Dorokhov D, Ignatov A, Deineko E, Serjapin A, Ala A and Skryabin K, 2004. Potential for gene flow from herbicide-resistant GM soybeans to wild soya in the Russian Far East. In: den Nijs HCM, Bartsch D and Sweet J (eds.). *Introgression from Genetically Modified Plants into Wild Relatives*. CAB International, Amsterdam, the Netherlands. pp. 151–161.

Duranti M and Gius C, 1997. Legume seeds: protein content and nutritional value. Field Crops Research, 53, 31–45.
EFSA (European Food Safety Authority), 2006. Guidance document for the risk assessment of genetically modified plants and derived food and feed by the Scientific Panel on Genetically Modified Organisms (GMO) – including draft document updated in 2008 (reference EFSA-Q-2003-005A). EFSA Journal 2006;4(4):99, 105 pp. doi:10.2903/j.efsa.2006.99
EFSA (European Food Safety Authority), 2007. Opinion of the Scientific Panel on genetically modified organisms (GMO) on an application (Reference EFSA-GMO-NL-2005-18) for the placing on the market of the glufosinate tolerant soybean A2704-12, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience. EFSA Journal 2007;5(7):524, 22 pp. doi:10.2903/j.efsa.2007.524
EFSA (European Food Safety Authority), 2009a. Statement of EFSA on the consolidated presentation of the joint Scientific Opinion of the GMO and BIOHAZ Panels on the "Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants" and the Scientific Opinion of the GMO Panel on "Consequences of the Opinion on the Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants on Previous EFSA Assessments of Individual GM Plants". EFSA Journal 2009;8(6):1108, 107 pp. doi:10.2903/j.efsa.2009.1108
EFSA (European Food Safety Authority), 2009b. Scientific Opinion of the Panel on Genetically Modified Organisms on an application (Reference EFSA-GMO-NL-2008-51) for the placing on the market of glyphosate tolerant genetically modified cotton GHB614, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience. EFSA Journal 2009;7(3):985, 24 pp. doi:10.2903/j.efsa.2009.985
EFSA (European Food Safety Authority), 2015. Explanatory note on DNA sequence similarity searches in the context of the assessment of horizontal gene transfer from plants to microorganisms. EFSA supporting publication 2015:EN-916, 10 pp.
EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010a. Statistical considerations for the safety evaluation of GMOs. EFSA Journal 2010;8(1):1250, 59 pp. doi:10.2903/j.efsa.2010.1250
EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010b. Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal 2010;8(7):1700, 168 pp. doi:10.2903/j.efsa.2010.1700
EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010c. Guidance on the environmental risk assessment of genetically modified plants. EFSA Journal 2010;8(11):1879. 111 pp. doi:10.2903/j.efsa.2010.1879
EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2011a. EFSA Panel on Genetically Modified Organisms (GMO); Scientific Opinion on Guidance for risk assessment of food and feed from genetically modified plants. EFSA Journal 2011;9(5):2150, 37 pp. doi:10.2903/j.efsa.2011.2150
EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2011b. Guidance on the post-market environmental monitoring (PMEM) of genetically modified plants. EFSA Journal 2011;9(8):2316, 40 pp. doi:10.2903/j.efsa.2011.2316
EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2011c. Scientific Opinion on application (EFSA-GMO-NL-2008-52) for the placing on the market of herbicide tolerant genetically modified soybean A5547-127 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience. EFSA Journal 2011;9(5):2147, 27 pp. doi:10.2903/j.efsa.2011.2147
EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2013. Scientific opinion on applications EFSA-GMO-RX-T25 and EFSA-GMO-NL-2007-46 for the renewal of authorisation of maize T25,1 and for the placing on the market of herbicide-tolerant genetically modified maize T25,2 both for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience AG. EFSA Journal 2013;11(10):3356, 30 pp. doi:10.2903/j.efsa.2013.3356
EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2015. Scientific Opinion on an application (EFSA-GMO-BE-2011-98) for the placing on the market of herbicide-tolerant genetically modified soybean FG72 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience. EFSA Journal 2015;13(7):4167, 29 pp. doi:10.2903/j.efsa.2015.4167
EFSA Scientific Committee, 2011. Guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed. EFSA Journal 2011;9(12):2438, 21 pp. doi:10.2903/j.efsa.2011.2438
Fasina YO, Classen HL, Garlich JD, Swaisgood HE and Clare DA, 2003. Investigating the possibility of monitoring lectin levels in commercial soybean meals intended for poultry feeding using steam-heated soybean meal as a model. Poultry Science, 82, 648–656.
Fernandez A, Mills EN, Lovik M, Spoek A, Germini A, Mikalsen A and Wal JM, 2013. Endogenous allergens and compositional analysis in the allergenicity assessment of genetically modified plants. Food and Chemical Toxicology, 62, 1–6.
Gandhi AP, 2009. Quality of soybean and its food products. International Food Research Journal, 16, 11–19.
Grant G, Dorward P, Buchan W, Armour J and Pusztai A, 1995. Consumption of diets containing raw soya beans (*Glycine max*), kidney beans (*Phaseolus vulgaris*), cowpea (*Vigna unguiculata*), or lupin seeds (*Lupinus angustifolius*) by rats for up to 700 days: effects on body composition and organ weights. British Journal of Nutrition, 73, 17–29.
Gumisiriza G and Rubaihayo PR, 1978. Factors that influence outcrossing in soybean. Zeitschrift für Acker- und Pflanzenbau/Journal of Agronomy and Crop Science, 147, 129–133.

Gupta YP, 1987. Anti-nutritional and toxic factors in food legumes: a review. Plant Foods for Human Nutrition, 37, 201–228.
Hart MM, Powell JR, Gulden RH, Levy-Booth DJ, Dunfield KE, Pauls KP, Swanton CJ, Klironomos JN and Trevors JT, 2009. Detection of transgenic *cp4 epsps* genes in the soil food web. Agronomy for Sustainable Development, 29, 7–501.
Hymowitz T, Singh RJ and Kollipara KP, 1998. The genomes of the *Glycine*. Plant Breeding Reviews, 16, 289–317.
Kikuchi A, Murata K, Tabuchi K and Sakai S, 1993. Inheritance of seed embryo color and investigation of degree of natural cross-pollination in soybeans. Breeding Science, 43, 112.
König A, Cockburn A, Crevel RW, Debruyne E, Grafstroem R, Hammerling U, Kimber I, Knudsen I, Kuiper HA, Peijnenburg AA and Penninks AH, 2004. Assessment of the safety of foods derived from genetically modified (GM) crops. Food and Chemical Toxicology, 42, 1047–1088.
Lajolo FM and Genovese MI, 2002. Nutritional significance of lectins and enzyme inhibitors from legumes. Journal of Agricultural and Food Chemistry, 50, 6592–6598. doi:10.1021/jf020191k.
Lecoq E, Holt K, Janssens J, Legris G, Pleysier A, Tinland B and Wandelt C, 2007. General surveillance: roles and responsibilities the industry view. Journal of Consumer Protection and Food Safety, 2, 25–28.
Lee B, Kim C-G, Park J-Y, Woong Park K, Kim H-J, Yi H, Jeong S-C, Kee Yoon W and Mook Kim H, 2009. Monitoring the occurrence of genetically modified soybean and maize in cultivated fields along the transportation routes of the Incheon Port in South Korea. Food Control, 20, 250–254.
Liener IE, 1955. The photometric determination of the hemagglutinating activity of soyin and crude soybean extracts. Archives of biochemistry and biophysics, 54, 223–231.
Liener IE, 1994. Implications of antinutritional components in soybean foods. Critical Reviews in Food Science & Nutrition, 34, 31–67.
Lu BR, 2005. Multidirectional gene flow among wild, weedy, and cultivated soybeans. In: Gressel J (ed.), *Crop Ferality and Volunteerism*, OECD + Taylor & Francis, pp. 137–147.
Maenz DD, Irish GG and Classen HL, 1999. Carbohydrate-binding and agglutinating lectins in raw and processed soybean meals. Animal Feed Science and Technology, 76, 335–343.
Maria John KM, Khan F, Luthria DL, Garrett W and Natarajan S, 2017. Proteomic analysis of anti-nutritional factors (ANF's) in soybean seeds as affected by environmental and genetic factors. Food Chemistry, 218, 321–329. doi: 10.1016/j.foodchem.2016.09.072
Miyake K, Tanaka T and McNeil PL, 2007. Lectin-based food poisoning: a new mechanism of protein toxicity. PLoS ONE, 2, e687. doi: 10.1371/journal.pone.0000687
Nachbar MS and Oppenheim JD, 1980. Lectins in the United States diets: a survey of lectins in commonly consumed food and a review of the literature. American Journal of Clinical Nutrition, 33, 2338–2435.
Nakayama Y and Yamaguchi H, 2002. Natural hybridization in wild soybean (*Glycine max* ssp. *soja*) by pollen flow from cultivated soybean (*Glycine max* ssp. *max*) in a designed population. Weed Biology and Management, 2, 25–30.
Nasi A, Picariello G and Ferranti P, 2009. Proteomic approaches to study structure, functions and toxicity of legume seeds lectins. Perspectives for the assessment of food quality and safety. Journal of Proteomics, 72, 527–538.
Noah ND, Bender AE, Reaidi GB and Gilbert RJ, 1980. Food poisoning from raw red kidney beans. British Medical Journal, 281, 236–237.
NRC (National Research Council), 1994. Nutrient Requirements of Poultry, 9th ed.; Academy Press, Washington, DC.
OECD (Organisation for Economic Co-operation and Development), 2000. Consensus document on the biology of *Glycine max* (L.) Merr. (soybean). Series on Harmonization of Regulatory Oversight in Biotechnology ENV/JM/MONO(2000)9, No. 15, 1–20. Available online: http://www.olis.oecd.org/olis/2000doc.nsf/LinkTo/NT00002C3A/$FILE/00085953.PDF
OECD (Organisation for Economic Co-operation and Development), 2001. Consensus document on compositional considerations for new varieties of soybean: Key food and feed nutrients and anti-nutrients.Available online: www.oecd.org/dataoecd/15/60/46815135.pdf
OECD (Organisation for Economic Co-operation and Development), 2012. Revised consensus document on compositional considerations for new varieties of soybean [*Glycine max* (L.) *merr.*]: Key food and feed nutrients, anti-nutrients, toxicants and allergens.
Owen MDK, 2005. Maize and soybeans – Controllable volunteerism without ferality? In: Gressel J (ed.), *Crop Ferality and Volunteerism*, OECD + Taylor & Francis, pp. 149–165.
Peumans WJ and Van Damme EJM, 1996. Prevalence, biological activity and genetic manipulation of lectins in foods. Trends in Food Science and Technology, 7, 132–138.
Polevoda B and Sherman F, 2000. Nα-terminal acetylation of eukaryotic proteins. Journal of Biological Chemistry, 275, 36479–36482.
Ray JD, Kilen TC, Abel CA and Paris RL, 2003. Soybean natural cross-pollination rates under field conditions. Environmental Biosafety Research, 2, 133–138.
Reddy NR and Pearson ND, 1994. Reduction in antinutritional and toxic components in plant foods by fermentation. Food Research international, 27, 281–290. doi: 10.1016/0963-9969(94)90096-5

Rizzi C, Galeoto L, Zoccatelli G, Vincenzi S, Chignola R and Peruffo AD, 2003. Active soybean lectin in foods: quantitative determination by ELISA using immobilised asialofetuin. Food research international, 36, 815–821.
Rodhouse JC, Hough GA, Roberts D and Gilbert RJ, 1990. Red kidney bean poisoning in the UK: an analysis of 50 suspected incidents between 1976 and 1989. Epidemiology and Infection, 105, 485–491.
Sharon N and Lis H, 2004. History of lectins: from hemagglutinins to biological recognition molecules. Glycobiology, 14, 53R–62R.
Singh RJ, Kollipara KP and Hymowitz T, 1987. Intersubgeneric hybridization of soybeans with a wild perennial species, *Glycine clandestina* Wendl. Theoretical and Applied Genetics, 74, 391–396.
Vasconcelos IM and Oliveira JT, 2004. Antinutritional properties of plant lectins. Toxicon, 44, 385–403.
Warren RL, Freeman JD, Levesque RC, Smailus DE, Flibotte S and Holt RA, 2008. Transcription of foreign DNA in *Escherichia coli*. Genome Research, 18, 1799–1805.
Windels P, Alcalde E, Lecoq E, Legris G, Pleysier A, Tinland B and Wandelt C, 2008. General surveillance for import and processing: the EuropaBio approach. Journal of Consumer Protection and Food Safety, 3, 14–16.
Yoshimura Y, Matsuo K and Yasuda K, 2006. Gene flow from GM glyphosate-tolerant to conventional soybeans under field conditions in Japan. Environmental Biosafety Research, 5, 169–173.

Abbreviations

ANOVA	analysis of variance
AST	aspartate aminotransferase
BSA	bovine serum albumin
bw	body weight
DCO	detailed clinical observation
EC	European Commission
ELISA	enzyme-linked immunosorbent assay
ERA	environmental risk assessment
GLP	Good Laboratory Practice
GMO	genetically modified organism
HR	homologous recombination
LC–MS/MS	liquid chromatography–mass spectrometry/mass spectrometry
NDF	neutral detergent fibre
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PCR	polymerase chain reaction
PLT	platelet
RBC	red blood cell
RET	reticulocyte
UTR	untranslated region
WBC	white blood cell