SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 26 October 2016

doi: 10.2903/j.efsa.2016.4642

Scientific Opinion on an application by Dow AgroSciences (EFSA-GMO-NL-2013-116) for placing on the market of genetically modified insect-resistant soybean DAS-81419-2 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, Philippe Guerche, Huw Jones, Barbara Manachini, Antoine Messéan, Elsa Ebbesen Nielsen, Fabien Nogué, Christophe Robaglia, Nils Rostoks, Jeremy Sweet, Christoph Tebbe, Francesco Visioli, Jean-Michel Wal, Zoltán Divéki, Antonio Fernández-Dumont, Andrea Gennaro, Anna Lanzoni, Franco Maria Neri and Konstantinos Paraskevopoulos

Abstract

Soybean DAS-81419-2 was developed by *Agrobacterium tumefaciens*-mediated transformation. It expresses the Cry1F and Cry1Ac proteins to confer resistance to certain lepidopteran species and the PAT protein that confers tolerance to glufosinate ammonium-based herbicides and that was used as a selectable marker gene. The molecular characterisation of soybean DAS-81419-2 did not give rise to safety issues. The agronomic, phenotypic and compositional characteristics of soybean DAS-81419-2 tested under field conditions revealed no relevant differences between soybean DAS-81419-2 and its conventional counterpart that would give rise to any food and feed or environmental safety concerns. There were no concerns regarding the potential toxicity and allergenicity of the newly expressed proteins Cry1F, Cry1Ac and PAT, and no evidence that the genetic modification might significantly change the overall allergenicity of soybean DAS-81419-2. The nutritional value of soybean DAS-81419-2 is not expected to differ from that of non-GM soybean varieties and no post-market monitoring of food/feed is considered necessary. There are no indications of an increased likelihood of establishment and spread of occasional feral soybean DAS-81419-2 plants, unless these plants are exposed to glufosinate ammonium-based herbicides or infested by insect pests that are susceptible to the Cry1F and Cry1Ac proteins. This will not result in different environmental impacts compared to conventional soybean. Considering the scope of this application, interactions with the biotic and abiotic environment were not considered to be an issue. Risks associated with an unlikely but theoretically possible horizontal gene transfer from soybean DAS-81419-2 to bacteria have not been identified. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of soybean DAS-81419-2. The GMO Panel concludes that the soybean DAS-81419-2 is as safe and as nutritious as its conventional counterpart and the tested non-GM reference varieties in the context of its scope.

Keywords: GMO, soybean (*Glycine max*), DAS-81419-2, Cry1F, Cry1Ac, PAT, Regulation (EC) No 1829/2003, import and processing

Requestor: Competent Authority of the Netherlands

Question number: EFSA-Q-2013-00527

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.

Acknowledgements: The Panel wishes to thank 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, Guerche P, Jones H, Manachini B, Messéan A, Nielsen EE, Nogué F, Robaglia C, Rostoks N, Sweet J, Tebbe C, Visioli F, Wal J-M, Divéki Z, Fernández-Dumont A, Gennaro A, Lanzoni A, Neri FM and Paraskevopoulos K, 2016. Scientific Opinion on an application by Dow AgroSciences (EFSA-GMO-NL-2013-116) for placing on the market of genetically modified insect-resistant soybean DAS-81419-2 for food and feed uses, import and processing under Regulation (EC) No 1829/2003. EFSA Journal 2016;14(12):4642, 23 pp. doi:10.2903/j.efsa.2016.4642

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Summary

Following the submission of an application (EFSA-GMO-NL-2013-116) under Regulation (EC) No 1829/2003[1] from Dow AgroSciences LLC, the EFSA Panel on Genetically Modified Organisms (GMO Panel) was asked to deliver a scientific opinion on the safety of insect-resistant genetically modified (GM) soybean (*Glycine max* L.) DAS-81419-2 (Unique Identifier DAS-81419-2). The scope of application EFSA-GMO-NL-2013-116 is for import, processing, and food and feed uses of soybean DAS-81419-2 within the European Union (EU), but it excludes cultivation in the EU. Soybean DAS-81419-2 expresses Cry1F and Cry1Ac proteins which confer resistance to lepidopteran insect pests. Soybean DAS-81419-2 also expresses the *pat* gene producing the PAT protein that confers tolerance to glufosinate ammonium-based herbicides and that was used as a selectable marker gene only. Therefore, in line with the scope of the application, the tolerance to glufosinate ammonium-based herbicides was not assessed in the comparative analyses.

The GMO Panel evaluated soybean DAS-81419-2 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 and the post-market environmental monitoring plan (PMEM).

Soybean DAS-81419-2 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 Cry1F and Cry1Ac proteins which confer resistance to lepidopteran insect pests. Soybean DAS-81419-2 also produces the PAT protein which confers tolerance to glufosinate ammonium-based herbicides and was used as a selectable marker during product development. The molecular characterisation data established that soybean DAS-81419-2 contains a single insert consisting of the *cry1Fv3*, *cry1Ac* (*synpro*) and *pat* expression cassettes producing the Cry1F, Cry1Ac and the PAT proteins. No other parts of the plasmid used for transformation were detected in soybean DAS-81419-2. Bioinformatic analyses and genetic stability studies were performed and the results did not give rise to safety issues. The levels of the newly expressed proteins present in soybean DAS-81419-2 were obtained and reported adequately. The protein characterisation data comparing the structural, biochemical and functional properties of plant and microbial derived Cry1F, Cry1Ac and PAT proteins, indicate that these proteins are equivalent.

Based on the agronomic and phenotypic characteristics of soybean DAS-81419-2 tested under field conditions, none of the differences identified between soybean DAS-81419-2 and its conventional counterpart required further assessment, except for '100 seed weight'. Additionally, no relevant differences were observed between soybean DAS-81419-2 and its conventional counterpart with regard to seed germination when tested under controlled conditions.

The safety assessment identified no concerns regarding the potential toxicity and allergenicity of the proteins Cry1F, Cry1Ac and PAT, newly expressed in soybean DAS-81419-2, and found no evidence that the genetic modification might significantly change the overall allergenicity of soybean DAS-81419-2, when compared with that of its conventional counterpart and non-GM commercial reference soybean varieties. Based on the outcome of the comparative assessment, the nutritional value of food and feed derived from soybean DAS-81419-2 is not expected to differ from that of food and feed derived from non-GM soybean varieties. The GMO Panel considers that post-market monitoring of food/feed derived from soybean DAS-81419-2 is not necessary, given the absence of safety concerns identified. Application EFSA-GMO-NL-2013-116 covers the import, processing, and food/feed uses of soybean DAS-81419-2, excluding cultivation. Therefore, the environmental risk assessment is concerned with the accidental release into the environment of viable soybean DAS-81419-2 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).

Due to the low survival capacity of soybean, the observed difference in '100 seed weight' is unlikely to change the fitness (e.g. survival, fecundity, competitiveness) or invasiveness characteristics of soybean DAS-81419-2 plants. In the case of accidental release into the environment of viable seeds of soybean DAS-81419-2, there are no indications of an increased likelihood of establishment and spread

[1] 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.

of occasional feral soybean DAS-81419-2 plants, unless these plants are exposed to glufosinate ammonium-based herbicides or infested by insect pests that are susceptible to the Cry1F and Cry1Ac proteins. However, the GMO Panel is of the opinion that the latter will not result in different environmental impacts compared to conventional soybean. Considering the scope of the application EFSA-GMO-NL-2013-116, interactions with the biotic and abiotic environment are not considered to be relevant issues. Bioinformatic analysis of the inserted DNA identified sufficient sequence identity with bacterial DNA which could theoretically facilitate the transfer of a plant codon-optimised *cry* gene and a *pat* gene onto a plasmid of a soil bacterium. Based on the functional proteins encoded by the genes and the prevalence of natural variants of such genes in environmental bacterial communities, the GMO panel did not identify a concern in relation to horizontal gene transfer to bacteria. 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-81419-2 would not raise safety concerns in the event of accidental release of viable GM soybean seeds into the environment. The PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of soybean DAS-81419-2 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-2013-116, 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-81419-2 addresses the scientific comments raised by the Member States and that soybean DAS-81419-2, as described in this application and under current processing procedures, is as safe and nutritious as its conventional counterpart and the tested 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.

Table of contents

Abstract 1
Summary 4
1. Introduction 6
1.1. Background 6
1.2. Terms of Reference as provided by the requestor 6
2. Data and methodologies 7
2.1. Data 7
2.2. Methodologies 7
3. Assessment 7
3.1. Molecular characterisation 7
3.1.1. Evaluation of relevant scientific data 7
3.1.1.1. Transformation process and vector constructs 7
3.1.1.2. Transgene constructs in the GM plant 8
3.1.1.3. Protein characterisation and equivalence 8
3.1.1.4. Information on the expression of the insert 9
3.1.1.5. Inheritance and stability of inserted DNA 9
3.1.2. Conclusion on molecular characterisation 9
3.2. Comparative analyses 10
3.2.1. Evaluation of relevant scientific data 10
3.2.1.1. Choice of comparator and production of material for the comparative assessment 10
3.2.1.2. Agronomic and phenotypic characteristics 10
3.2.1.3. Compositional analysis 11
3.2.2. Conclusion on comparative analysis 12
3.3. Food/feed safety assessment 12
3.3.1. Evaluation of relevant scientific data 12
3.3.1.1. Effect of processing 12
3.3.1.2. Toxicology 12
3.3.1.3. Allergenicity 13
3.3.1.4. Nutritional assessment of GM food/feed 15
3.3.1.5. Post-market monitoring of GM food/feed 15
3.3.2. Conclusion on the food/feed safety assessment 15
3.4. Environmental risk assessment and monitoring plan 15
3.4.1. Evaluation of relevant scientific data 15
3.4.1.1. Environmental risk assessment 15
3.4.2. Post-market environmental monitoring 18
3.4.3. Conclusion on the environmental risk assessment and monitoring plan 19
4. Conclusions 19
Documentation provided to EFSA 19
References 21
Abbreviations 23

1. Introduction

The scope of application EFSA-GMO-NL-2013-116 is for import, processing, and food/feed uses of soybean DAS-81419-2 derived from grain and forage and does not include cultivation in the European Union (EU).[2]

Soybean DAS-81419-2 was developed to confer resistance to certain lepidopteran chewing pests. The resistance is achieved by the expression of the Cry1F and Cry1Ac proteins from *Bacillus thuringiensis*. Soybean DAS-81419-2 also expresses the PAT protein from *Streptomyces viridochromogenes*, that confers tolerance to glufosinate ammonium-based herbicides and that was used as a selectable marker gene.[3]

1.1. Background

On 13 May 2013, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands an application (Reference EFSA-GMO-NL-2013-116) for authorisation of GM soybean DAS-81419-2 (unique identifier DAS-81419-2), submitted by Dow AgroSciences LLC within the framework of Regulation (EC) No 1829/2003 on GM food and feed.

After receiving the application EFSA-GMO-NL-2013-116, and in accordance with Articles 5(2)(b) and 17(2)(b) of the Regulation (EC) No 1829/2003, EFSA informed the Member States and the European Commission, and made the summary of the application publicly available on the EFSA website.[4] EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of the Regulation (EC) No 1829/2003. On 21 November 2013 and 17 January 2014, EFSA received additional information requested under completeness check 25 June 2013 and 12 December, respectively. On 7 February 2014, 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 the Member States and the European Commission, and consulted nominated risk assessment bodies of the Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC[5] following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. The Member States had 3 months after the date of receipt of the valid application (until 27 May 2014) to make their opinion known.

On 12 February 2014 and 25 April 2016 (European Union Reference Laboratories-Joint Research Centre (EURL-JRC)), 8 April 2014, 28 May 2014, 23 June 2014, 9 September 2014, 1 October 2015, 23 November 2015, 17 February 2016, 15 March 2016, 26 May 2016, 10 June 2016 and 10 August 2016, the EFSA Panel on Genetically Modified Organisms (GMO Panel) requested additional information from the applicant. The applicant provided the requested information on 20 February 2014 and 7 September 2016 (EURL-JRC), 18 June 2014, 27 August 2014, 12 November 2014, 25 November 2014, 24 November 2015, 18 January 2016, 7 March 2016, 29 March 2016, 13 June 2016, 17 June 2016 and 1 September 2016. The applicant also spontaneously provided additional information on 16 December 2014, 18 January 2016, 22 January 2016 and 31 March 2016.

In giving its scientific opinion on soybean DAS-81419-2 to the European Commission, the Member States and the applicant, and in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003, EFSA has endeavoured to respect a time limit of 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.

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 EFSA GMO Panel was requested to carry out a scientific assessment of soybean DAS-81419-2 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003.

[2] Additional information: 22/1/2016.
[3] Dossier: Part II – Section A2.
[4] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2013-00527
[5] 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.

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 genetically modified organisms (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 EFSA Panel took into account application EFSA-GMO-NL-2013-116, 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 a scientific risk assessment of soybean DAS-81419-2 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), for the environmental risk assessment (ERA) of GM plants (EFSA GMO Panel, 2010a) and for the post-market environmental monitoring (PMEM) of GM plants (EFSA GMO Panel, 2011b).

The comments raised by the Member States are addressed in Annex G of EFSA's overall opinion[4] and were taken into consideration during the scientific 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

Soybean DAS-81419-2 was developed by *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*)-mediated transformation of cotyledonary nodes of soybean (*Glycine max* (L.) Merr.) variety Maverick with the *A. tumefaciens* strain EHA101 containing the binary plasmid pDAB9582. The transfer-deoxyribonucleic acid (T-DNA) present in plasmid pDAB9582 consisted of the following elements between their respective right and left border (border A and B, respectively) regions: partial MAR (matrix attachment region) sequence from *Nicotiana tabacum* rb-7-5A gene; AtUbi10 promoter, 5′ untranslated region (UTR) and intron from *Arabidopsis thaliana* polyubiquitin 10 (*UBQ10*) gene to direct constitutive expression; synthetic *cry1Fv3* coding sequence producing the chimeric Cry1F protein and consisting of parts of the *cry1Fa2* and *cry1Ca3* genes from *B. thuringiensis* subsp. *aizawai* strain PS811 and part of the *cry1Ab1* gene from *B. thuringiensis* subsp. *berliner* strain 1715; 3′ UTR of the open reading frame (ORF) 23 from the *A. tumefaciens* plasmid pTi15955 that terminates transcription and directs polyadenylation; promoter and 5′ UTR from *Cassava vein mosaic virus* (CsVMV) to drive constitutive expression; synthetic *cry1Ac* (*synpro*) coding sequence producing the chimeric Cry1Ac protein and consisting of a part of the *cry1Ac1* gene from *B. thuringiensis* subsp. *kurstaki* strain HD73, part of the *cry1Ca3* gene from *B. thuringiensis* subsp. *aizawai* strain PS811 and part of the *cry1Ab1* gene from *B. thuringiensis* subsp. *berliner* strain 1715; 3′ UTR of the AtuORF23 from the *A. tumefaciens* plasmid pTi15955 that terminates transcription and directs polyadenylation; promoter and 5′ UTR from CsVMV to direct constitutive expression; a *pat* coding sequence from *S. viridochromogenes*; 3′ UTR of the AtuORF1 from the *A. tumefaciens* plasmid pTi15955 that terminates transcription and directs polyadenylation.

Additional functional elements in the plasmid vector outside the T-DNA, and thus not expected to be transferred to the soybean genome, were: *ori* origin of replication from broad host range RK2

plasmid, for the maintenance of pDAB9582 in bacteria; *trfA* from the RK2 plasmid serving as replication initiator; *SpecR* bacterial selectable marker gene from *Escherichia coli* Tn7 transposon to confer spectinomycin resistance.

3.1.1.2. Transgene constructs in the GM plant

Molecular characterisation of soybean DAS-81419-2 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.[6] The approach used was acceptable both in terms of coverage and sensitivity.

Southern analyses indicated that soybean DAS-81419-2 contains a single insert, which consists of a single copy of the T-DNA in the same configuration as in the pDAB9582 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 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-81419-2 together with 1,297 bp of the 5′ and 1,379 bp of the 3′ flanking regions were determined. The insert of 12,496 bp is identical to the T-DNA of pDAB9582, except for the deletion of 23 bp and 408 bp of the 5′ and 3′ border regions, respectively. In addition, 37 bp of filler DNA and a 98 bp fragment of *cry1Ac* (*synpro*) were inserted at the 5′ junction and 9 bp of filler DNA were inserted at the 3′ junction of the insert during the integration process. A comparison with the pre-insertion locus indicated that 57 bp were deleted from soybean genomic DNA. The possible interruption of known endogenous soybean genes by the insertion in event DAS-81419-2 was evaluated by bioinformatic analyses of the pre-insertion locus and of the genomic sequences flanking the insert. The results from these analyses did not indicate the interruption of any known endogenous gene in soybean DAS-81419-2.[7]

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 three newly expressed proteins Cry1F, Cry1Ac and PAT revealed no significant similarities to toxins and allergens.[7] In addition, updated bioinformatics analyses of the newly created ORFs within the inserts and at their junctions indicate that the expression of an ORF showing significant similarities to toxins or allergens is highly unlikely.[7]

In order to assess the possibility for horizontal gene transfer (HGT) by homologous recombination (HR), the applicant performed a sequence identity analysis of the regions of bacterial origin in soybean DAS-81419-29. Three elements were identified with sufficient length and identity to support HR (de Vries and Wackernagel, 2002; Monier et al., 2007; Hülter and Wackernagel, 2008; EFSA, 2009; Overballe-Petersen et al., 2013), namely a 709 bp fragment of *A. tumefaciens* Ti plasmid pTi15955 containing the 3′ untranslated region of AtuORF1 and two fragments of 526 and 457 bp, respectively, from the same plasmid and containing the 3′ untranslated region of AtuORF23. A double HR event involving 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-81419-2 expresses three new proteins, Cry1F, Cry1Ac and PAT

Given the technical restraints in producing large enough protein quantities for safety testing from plants, these proteins were recombinantly produced in microbes, *Pseudomonas fluorescens* or *E. coli*. Prior to safety studies, a panel of biochemical methods were employed to demonstrate the equivalence between soybean and microbe-derived proteins. Purified proteins from these sources were characterised and compared in terms of their physicochemical, structural and functional properties.[8,9]

Cry1F characterisation and equivalence. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis showed that full-length plant and microbe-derived Cry1F proteins had the expected molecular weight of ~ 130 kDa and were comparably immunoreactive to Cry1F protein specific antibodies. Glycosylation detection analysis demonstrated that none of the Cry1F

[6] Dossier: Part II – Section A2.1.
[7] Additional information: 18/1/2016.
[8] Additional information: 29/3/2016.
[9] Dossier: Part II – Section A4.2.

proteins were N-glycosylated. Amino acid sequence analysis by mass spectrometry methods showed that both proteins matched their expected sequence. Functional equivalence was demonstrated by an insect feeding bioassay which showed that both proteins had comparable insecticidal activity.

Cry1Ac characterisation and equivalence. SDS-PAGE and western blot analysis showed that full-length plant and microbe-derived Cry1Ac proteins had the expected molecular weight of ~ 130 kDa and were comparably immunoreactive to Cry1Ac protein specific antibodies. Glycosylation detection analysis demonstrated that none of the Cry1Ac proteins were N-glycosylated. Amino acid sequence analysis by mass spectrometry methods showed that both proteins matched their expected sequence. Functional equivalence was demonstrated by an insect feeding bioassay which showed that both proteins had comparable insecticidal activity.

PAT characterisation and equivalence. SDS-PAGE and western blot analysis showed that plant and microbe-derived PAT proteins had the expected molecular weight of ~ 20 kDa and were comparably immunoreactive to PAT protein specific antibodies. Glycosylation detection analysis demonstrated that none of the PAT proteins were N-glycosylated. Amino acid sequence analysis by mass spectrometry methods showed that both proteins matched their expected sequence. Functional equivalence was demonstrated by a biochemical *in vitro* activity assay which showed that both proteins had comparable activity and specificity for the intended herbicide.

Based on this, the GMO Panel accepts the use of the Cry1F, Cry1Ac and PAT proteins derived from *P. fluorescens* or *E. coli* in the safety studies.

3.1.1.4. Information on the expression of the insert

Levels of the Cry1F, Cry1Ac and PAT proteins were analysed by enzyme-linked immunosorbent assay (ELISA) in the leaf, root, forage and seed of soybean DAS-81419-2 in 2011 from 10 locations in the USA.[10] Considering the scope of the application, the Cry1F, Cry1Ac and PAT protein levels in seed are considered the most relevant. The mean values and ranges of protein expression levels in seed (n = 40) are summarised in Table 1.

Table 1: Means, standard deviations and ranges of protein levels in (μg/g dry weight) from soybean DAS-81419-2 seeds (number of samples is 40)

Tissue	Cry1F	Cry1Ac	PAT
Seed	13.8[(a)] ± 1.24[(b)]	1.04 ± 0.1	0.86 ± 0.13
	(10.41–16.95)[(c)]	(0.79–1.40)	(0.63–1.12)

(a): Mean.
(b): Standard deviation.
(c): Range.

3.1.1.5. Inheritance and stability of inserted DNA[11]

Genetic stability of the soybean DAS-81419-2 insert was assessed by Southern analysis of genomic DNA from five different 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, PAT protein expression and event-specific PCR data. The results supported the presence of a single insertion, segregating in a Mendelian fashion.

3.1.2. Conclusion on molecular characterisation

The molecular characterisation data establish that soybean DAS-81419-2 contains a single insert consisting of one copy of the *Cry1Fv3*, *Cry1Ac* (*synpro*) and *pat* expression cassettes producing the Cry1F, Cry1Ac and PAT proteins, respectively. Bioinformatic analyses of the newly expressed proteins and the ORFs within the insert or spanning the junction sites between the insert and genomic DNA did not give rise to safety issues. The stability of the inserted DNA and of the introduced insect resistance and herbicide tolerance traits were confirmed over several generations. The levels of the Cry1F, Cry1Ac and PAT proteins were obtained and reported adequately. The protein characterisation data comparing the structural, biochemical and functional properties of plant and microbial derived Cry1F, Cry1Ac and PAT proteins, indicate that these proteins are equivalent.

[10] Dossier: Part II – Section A2.2.3.
[11] Dossier: Part II – Section A2.2.4.

3.2. Comparative analyses

3.2.1. Evaluation of relevant scientific data

3.2.1.1. Choice of comparator and production of material for the comparative assessment[12]

Application EFSA-GMO-NL-2013-116 presents data on agronomic and phenotypic characteristics, as well as forage and seed composition of soybean DAS-81419-2 derived from field trials performed at 11 sites in the USA in 2011 and 2012 (Table 2).

Field trials for the agronomic, phenotypic and compositional assessment of soybean DAS-81419-2 were conducted in major soybean growing areas of the USA,[13] representing regions of diverse agronomic practices and environmental conditions. At each site, the following materials were grown in a randomised complete block design with four replicates: soybean DAS-81419-2, the conventional counterpart and three non-GM soybean reference varieties; all treated (sprayed) with required maintenance pesticides (including conventional herbicides) according to local requirements. In total, nine non-GM soybean reference varieties[14] were included across all the field trials sites.

Soybean DAS-81419-2 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-81419-2 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 the line of soybean DAS-81419-2. The GMO Panel considers that this non-transgenic line is the appropriate conventional counterpart.

Table 2: Overview of comparative assessment studies with soybean DAS-81419-2 provided in application EFSA-GMO-NL-2013-116

Study focus	Study details	Comparator	Commercial reference varieties
Agronomic and phenotypic characteristics; composition	Field trials, 2011 and 2012, USA (11 locations)	Maverick	Nine non-GM varieties
Seed germination	Controlled conditions (warm and cold treatment)	Near-isogenic non-GM line	None

Statistical analysis of field trials data

The statistical analysis of the agronomic, phenotypic and compositional data from the 2011 and 2012 field trials followed the recommendations of the GMO Panel (EFSA GMO Panel, 2010b, 2011a). This includes 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 commercial 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 characteristics[16]

Agronomic and phenotypic characteristics tested under field conditions

The agronomic and phenotypic characteristics evaluated on the basis of data collected from the 11 field trial sites in the USA in 2011 and 2012 (Table 2) were: early population (stand count), seedling vigour, days to 50% flowering, disease incidence, insect damage, days to maturity, lodging, plant height, final population (stand count), number of seeds, number of pods, pod shattering, yield and '100 seed weight'. Data for four endpoints[17] were considered not suitable for a parametric analysis

[12] Dossier: Part II – Section A3; additional information: 27/8/2014.
[13] The sites in 2011 field trials were in Richland, IA; Atlantic, IA; Carlyle, IL; Wyoming, IL; Frankfort, IN; Fisk, MO; La Plata, MO; York, NE; Brunswick, NE; and Germansville, PA. The site of the 2012 field trial was in Sheridan, IN.
[14] The non-GM reference varieties were Pioneer 93M62, IL3503, DSR 75213-72, Porter 75148, HiSOY 38C60, Williams 82, Pioneer 93Y41, L&M 34 and Stine 3099-2.
[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] Dossier: Part II – Section A3.4; additional information 25/11/2014.
[17] Disease incidence, insect damage, lodging and seedling vigour.

because of the nature of the measurements: for those, differences between the GM soybean and the conventional counterpart were investigated with the Wilcoxon Signed Rank (WSR) test. Pod shattering was not analysed statistically because all the values were zero.

Statistically significant differences were identified between soybean DAS-81419-2 and its conventional counterpart for the endpoints days to 50% flowering, early stand count, plant height, number of pods, number of seeds, '100 seed weight' and yield. Except for '100 seed weight', all the endpoints fell within the equivalence limits established by the non-GM reference varieties (equivalence category I). For the endpoint '100 seed weight' equivalence was more likely than non-equivalence (equivalence category II). The endpoint 'final population' was not categorised for equivalence due to the small variation among the non-GM reference varieties, however, it was found not significantly different from the conventional counterpart. Within the endpoints analysed with the WSR test, statistically significant differences were identified for plant lodging and seedling vigour; however, in both cases the mean values for the GM soybean were within the range of the non-GM reference varieties.

Agronomic and phenotypic characteristics tested under controlled conditions[18]

Seed germination of soybean DAS-81419-2 was compared with that of its near-isogenic non-GM line under warm and cold conditions. 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 of the seeds to a constant temperature of 25°C for 5 days, while the cold treatment consisted of exposure to 10°C for 7 days followed by exposure to 25°C for 5 days. The germination rate of soybean DAS-81419-2 seeds under warm and cold conditions did not differ significantly from that of its conventional counterpart.

3.2.1.3. Compositional analysis[19]

Soybean forage and seeds harvested from the field trials in the USA in 2011 and 2012 (Table 2) were analysed for 87 different constituents (9 in forage and 78 in seed), including the key constituents recommended by the OECD (OECD, 2001).[20] For 17 seed constituents,[21] more than 50% of the observations were below the limit of quantification. The statistical analysis was applied to the remaining 70 constituents (9 in forage[22] and 61 in seed[23]).

For four seed endpoints,[24] equivalence could not be determined because of the small variation among the non-GM reference varieties. Three of those endpoints were found significantly different between soybean DAS-81419-2 and the conventional counterpart and were further considered (Table 3).

The combination of test of difference and test of equivalence could be applied to the remaining 66 endpoints. Statistically significant differences between soybean DAS-81419-2 and the conventional counterpart were identified for 23 of the endpoints (ash in forage and 22 seed endpoints[25]). All the endpoints fell under equivalence category I except linoleic acid (18:2) which fell under category IV (Table 3).

---

[18] Additional information: 27/8/2014.

[19] Dossier: Part II – Section A3 and study 130989; additional information: 25/11/2014, 24/11/2015, 7/3/2016, 13/6/2016 and 1/9/2016.

[20] The applicant showed that the method used for the analysis of lectin levels was reliable in quantifying relative changes in levels. Additional information: 7/3/2016, 13/6/2016 and 1/9/2016.

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

[22] The forage constituents included proximates (protein, fat, ash, moisture and carbohydrates), fibre (acid detergent fibre (ADF) and neutral detergent fibre (NDF)) and minerals (calcium and phosphorus).

[23] The seed constituents included proximates (protein, fat, ash, moisture and carbohydrates), fibre (ADF, NDF and total dietary fibre), amino acids (alanine, lysine, arginine, methionine, aspartic acid, phenylalanine, cystine, proline, glutamic acid, serine, histidine, tryptophan, leucine, valine, isoleucine, tyrosine, glycine and threonine), fatty acids (palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), arachidic (20:0), eicosenoic (20:1) and behenic (22:0)), vitamins (ascorbic acid, thiamine HCl, riboflavin, niacin, pantothenic acid, pyridoxine HCl, folic acid, α-tocopherol, γ-tocopherol and δ-tocopherol), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, selenium and zinc) and other compounds (total daidzein equivalent, total genistein equivalent, total glycitein equivalent, lectin, phytic acid, raffinose, stachyose and trypsin inhibitor).

[24] Selenium, aspartic acid, lysine and eicosenoic acid (20:1).

[25] Ash, crude protein, moisture, total fat, phenylalanine, palmitic acid (16:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), arachidic acid (20:0), behenic acid (22:0), phosphorus, potassium, zinc, thiamine HCl, niacin, pantothenic acid, ascorbic acid, α-tocopherol, γ-tocopherol, δ-tocopherol and total glycitein equivalent.

Table 3: Compositional endpoints from seed 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 the 2011 and 2012 field trials (Table 2)

Endpoint	Soybean DAS-81419-2	Conventional counterpart	Equivalence limits from non-GM reference varieties
Aspartic acid (% AA)	11.49*	11.52	–
Lysine (% AA)	6.426*	6.312	–
Linoleic acid (18:2) (% FA)	53.92*	54.23	(54.69, 56.84)
Eicosenoic acid (20:1) (% FA)	0.1197*	0.1323	–

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 (the equivalence test was not performed) and dark grey (equivalence category IV). % AA: percentage of total amino acids; % FA: percentage of total fatty acids; –: test of equivalence not applied due to the small variation among the non-GM reference varieties.

The GMO Panel assessed all the compositional differences between soybean DAS-81419-2 and its conventional counterpart. After considering the well-known biological role of the compounds and the magnitude of the changes observed, the GMO Panel did not identify any need for further food/feed safety assessment.

3.2.2. Conclusion on comparative analysis

The GMO Panel concludes that none of the differences identified in forage and seed composition between soybean DAS-81419-2 and its 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-81419-2 tested under field conditions, none of the differences identified between soybean DAS-81419-2 and its conventional counterpart requires further assessment, except for '100 seed weight'. Additionally, no relevant differences were observed between soybean DAS-81419-2 and its conventional counterpart with regard to seed germination tested under controlled conditions. The difference observed in '100 seed weight' between soybean DAS-81419-2 and its conventional counterpart is further assessed for its 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. Effect of processing[26]

Processed products

Based on the outcome of the comparative assessment, processing of soybean DAS-81419-2 into food and feed products is not expected to result in products being different from those of commercial non-GM soybean varieties.

Newly expressed proteins

The effect of heat treatment on Cry1F and Cry1Ac has been previously assessed by the GMO Panel (EFSA GMO Panel, 2010c). It was showed that Cry1F and Cry1Ac lost their insecticidal activity in a bioassay after being heated at 75 and 90°C and at pH = 7.5 for 30 min. In the context of this application, PAT protein solutions were heated (25–95°C) for 30 min. The PAT protein lost 99% of its enzymatic activity at ≥ 55°C, and 91% of its immunoreactivity at temperatures ≥ 37°C.

3.3.1.2. Toxicology

Toxicological assessment of newly expressed proteins[27]

The three proteins (Cry1F, Cry1Ac and PAT) newly expressed in soybean DAS-81419-2 have been extensively characterised and showed the expected molecular weight, immunoreactivity vs specific

[26] Dossier: Part II – Section A3.5.
[27] Dossier: Part II – Section A4.2; additional information 17/6/2016.

antibodies, amino acid sequence and functional activity (Section 3.1.1.3). Cry1F and Cry1Ac are delta-endotoxins with highly specific insecticidal properties. These proteins act through cellular receptors found in target insect species and it is reported that the gastrointestinal tract of mammals, including humans, lacks receptors with specific high affinity to Cry proteins (Hammond et al., 2013; Koch et al., 2015). The same Cry1F and Cry1Ac proteins and the PAT protein have been previously assessed by the GMO Panel (e.g. EFSA, 2007; EFSA GMO Panel, 2010c, 2011c) and no safety concerns for humans and animals were identified in the context of the applications assessed. Updated bioinformatic studies confirmed the absence of relevant similarities between these newly expressed proteins to known toxins (Section 3.1.1.2).

The applicant provided studies on Cry1F, Cry1Ac and PAT proteins from bacterial recombinant systems, which were considered equivalent to the plant-produced proteins (Section 3.1.1.3).

In vitro *degradation studies*[28]

The resistance to degradation by pepsin of the bacterial Cry1F, Cry1Ac and PAT proteins was investigated in solutions at pH ~ 1.2 in three independent studies. The studies on Cry1F and Cry1Ac have been previously assessed by the GMO Panel (EFSA GMO Panel, 2010c). In the context of the application EFSA-GMO-NL-2013-116, the applicant provided a study on the PAT protein where its integrity 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 PAT protein was degraded by pepsin within 1 min.

Acute oral toxicity testing[29]

A mixture of Cry1F and Cry1Ac proteins given to mice by gavages in amounts of 375 and 350 mg/kg body weight, respectively, did not induce effects attributable to the Cry proteins, neither did PAT administered at a single dose of 5,000 mg/kg. These studies have been previously assessed by the EFSA GMO Panel (2010c). In the context of this application, the applicant provided additional acute oral toxicity studies in mice, respectively, on a Cry1F protein at 300 mg/kg body weight; on a Cry1Ac protein at 700 mg/kg; and on a PAT protein at 2,000 mg/kg body weight. No effects attributable to these proteins were seen. The GMO Panel considers acute toxicity testing of the newly expressed proteins of little additional value to the risk assessment of the repeated human and animal consumption of food and feed derived from GM plants (EFSA GMO Panel, 2011a).

Toxicological assessment of components other than newly expressed proteins

Soybean DAS-81419-2 did not show any compositional difference to its conventional counterpart that would require further assessment (Section 3.2.1.3). No further food and feed safety assessment of components other than newly expressed proteins is required.

Animal studies with the food/feed derived from GM plants

No animal studies with food/feed derived from soybean DAS-81419-2 were provided by the applicant (e.g. 90-day toxicity studies in rodents or feeding studies in young rapidly growing animal species).

No substantial modifications in the composition of the food/feed derived from soybean DAS-81419-2 (see Section 3.2.1.3) were noted and no indication of possible unintended effects requiring further assessment were identified. Therefore, no animal studies on the food/feed derived from soybean DAS-81419-2 are required for the assessment of food/feed derived from soybean DAS-81419-2 (EFSA GMO Panel, 2011a).

3.3.1.3. 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[30]

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 yield sufficient evidence to predict allergenicity (Codex Alimentarius, 2009; EFSA GMO Panel, 2011a).

[28] Dossier: Part II – Section A5.1; additional information: 17/6/2016.
[29] Dossier: Part II – Section A4; additional information: 17/6/2016.
[30] Dossier: Part II – Section A5.1, A5.3; additional information: 17/6/2016; 18/1/2016.

The *cry1Fv3* and *cry1Ac* (*synpro*) genes originate from *B. thuringiensis*, while the *pat* gene originates from *S. viridochromogenes*, microorganisms which are not considered to be allergenic sources.

Updated bioinformatic analyses[7] of the amino acid sequences of the Cry1F, Cry1Ac 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 Cry1F, Cry1Ac and PAT proteins and known allergens, which confirmed the outcome of the previous bioinformatic analysis.

The study on resistance to degradation of the Cry1F, Cry1Ac and PAT proteins by pepsin has been described in Section 3.3.1.2.

The GMO Panel has previously evaluated the safety of the Cry1F, Cry1Ac and PAT proteins in the context of previous applications and no concerns on allergenicity were identified (e.g. EFSA GMO Panel, 2010c).

Proteins derived from *B. thuringiensis* (Bt proteins) have been suggested to possess adjuvant activity, based on animal studies on Cry1Ac (e.g. Vazquez et al., 1999). The Panel has previously evaluated the safety of the Cry1F and Cry1Ac proteins and no concerns on adjuvanticity in the context of the applications assessed were identified (EFSA GMO Panel, 2010c). From the limited experimental evidence available, the GMO Panel did not find indications that the presence of the Bt proteins at the levels expressed in soybean DAS-81419-2 might act as adjuvants with the potential to enhance a specific immunoglobulin E (IgE) response and to favour the development of an allergic reaction.

In the context of the present application, the GMO Panel considers that there are no indications that the newly expressed Cry1F, Cry1Ac and PAT proteins, individually or their simultaneous presence, in soybean DAS-81419-2 may be allergenic.

Assessment of allergenicity of the whole GM plant[31]

Soybean is considered to be a common allergenic food[32] (OECD, 2012). Therefore, any potential change in the endogenous allergenicity of the GM plant when compared with that of its comparator(s) should be assessed (EFSA GMO Panel, 2011a). The applicant performed *in vitro* allergenicity studies using human sera as probes as well as a comparative analysis using measurements of specific known soybean allergens in extracts from soybean DAS-81419-2, its conventional counterpart and a set of non-GM commercial reference soybean varieties.

Initially, the applicant provided one-dimensional electrophoresis followed by western blot and an inhibition ELISA study using pooled sera from ten individuals allergic to soybean. In addition, the applicant performed two-dimensional electrophoresis followed by western blot analyses and inhibition ELISA studies using single sera 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 by densitometry. According to the applicant, minor variations in spot intensities, which are commonly seen in such studies, were observed between soybean DAS-81419-2 and its conventional counterpart.

Consequently, 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 been previously considered as an alternative/complementary acceptable approach for the assessment of endogenous allergenicity in GM plants (EFSA GMO Panel, 2010d; 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 OECD (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 an over-expression of natural endogenous allergen.

In the context of this application, the GMO Panel considers that there is no evidence that the genetic modification might significantly change the overall allergenicity of soybean DAS-81419-2 when compared with that of its conventional counterpart and non-GM commercial reference soybean varieties.

[31] Dossier: Part II – Section A5.2; additional information 18/1/16.

[32] 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.

3.3.1.4. Nutritional assessment of GM food/feed

The intended trait of soybean DAS-81419-2 is insect resistance, with no intention to alter nutritional parameters. Comparison of the composition of soybean DAS-81419-2 with its conventional counterpart and non-GM reference varieties did not identify differences that would require nutritional assessment. Based on these data, the nutritional value of food and feed derived from soybean DAS-81419-2 is not expected to differ from that of food and feed derived from non-GM soybean varieties.

3.3.1.5. Post-market monitoring of GM food/feed

No biologically relevant compositional, agronomic and phenotypic changes were identified in soybean DAS-81419-2 when compared with its conventional counterpart. Furthermore, the overall intake or exposure is not expected to change because of the introduction of soybean DAS-81419-2 into the market. The GMO Panel therefore considers soybean DAS-81419-2 to be as safe as its conventional counterpart and that post-market monitoring (EFSA GMO Panel, 2011a) of the food/feed derived from soybean DAS-81419-2 is not necessary.

3.3.2. Conclusion on the food/feed safety assessment

The safety assessment identified no concerns regarding the potential toxicity and allergenicity of the proteins Cry1F, Cry1Ac and PAT, expressed in soybean DAS-81419-2, and found no evidence that the genetic modification might significantly change the overall allergenicity of soybean DAS-81419-2 when compared with that of its conventional counterpart and non-GM commercial reference soybean varieties. Based on the outcome of the comparative assessment, the nutritional value of food and feed derived from soybean DAS-81419-2 is not expected to differ from that of food and feed derived from non-GM soybean varieties. The GMO Panel concludes that soybean DAS-81419-2 is as safe and nutritious as its conventional counterpart and non-GM soybean reference varieties tested.

3.4. Environmental risk assessment and monitoring plan

3.4.1. Evaluation of relevant scientific data

Considering the scope of application EFSA-GMO-NL-2013-116 (which excludes cultivation), the environmental risk assessment (ERA) of soybean DAS-81419-2 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 to their faecal material (manure and faeces); and (2) the accidental release into the environment of viable soybean DAS-81419-2 seeds during transportation and processing (EFSA GMO Panel, 2010a).

3.4.1.1. Environmental risk assessment

Persistence and invasiveness of the GM plant[33]

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 the absence of a dormancy phase, 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, the absence of a dormancy phase, and susceptibility to plant pathogens and cold climatic conditions.

The applicant presented agronomic and phenotypic data on soybean DAS-81419-2 gathered from field trials conducted in soybean growing areas in the USA (Section 3.2.1.2). The data showed relevant difference in '100 seed weight' which was reduced for soybean DAS-81419-2 seeds. No relevant differences in the other measured plant characteristics were identified (Section 3.2.2). Due to the low survival capacity of soybean, the observed difference in '100 seed weight' is unlikely to change the fitness (e.g. survival, fecundity, competitiveness) or invasiveness characteristics of soybean DAS-81419-2 plants.

[33] Dossier: Part II – Sections E3.1; additional information: 27/8/2014; 25/11/2014.

As the general characteristics of soybean DAS-81419-2 remain unchanged compared to its conventional counterpart, it is considered very unlikely that soybean DAS-81419-2 will differ from non-GM soybean varieties in its ability to survive until subsequent seasons, or to establish occasional feral plants under European environmental conditions in case of accidental release into the environment of viable soybean DAS-81419-2 seeds during transportation and processing.

The GMO Panel is not aware of any scientific report of increased survival capacity, including overwintering, of existing GM soybeans 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-81419-2 in Europe will not be different from that of non-GM soybean varieties.

Effects of gene transfer[34]

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through HGT of DNA, or through vertical gene flow via cross-pollination from flowering plants arising from spilled seed.

1) Plant-to-microorganism gene transfer

Genomic plant DNA is a component of several 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 plants to bacteria) is not likely to occur at detectable frequencies under natural conditions (EFSA, 2009).

A successful HGT 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 into bacterial genomes is homologous recombination. 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 event DAS-81419-2 contains several genetic elements of bacterial origin. These are: (1) the *cry1Fv3* (synthetic version of the *cry1F* gene from *B. thuringiensis* subsp. *aizawai* strain PS811); (2) the *cry1Ac* (*synpro*) (synthetic version of the *cry1Ac* gene from *B. thuringiensis* subsp. *kurstaki* strain HD73); (3) the *pat* (synthetic version of phosphinothricin acetyltransferase gene from *S. viridochromogenes*) and the (4) three untranslated regions (UTR) two AtuORF23 and one AtuORF1 of *A. tumefaciens* pTi15955.

Bioinformatic analysis of the inserted DNA confirmed that the plant codon-use optimised bacterial genes *cry1Fv3*, *cry1Ac* (*synpro*) and pat did not provide sufficient sequence identity to facilitate homologous recombination. However, sufficient sequence identity with bacterial DNA was found for the AtuORF23 3′ sequence, that flanks the *cry1Ac* (*synpro*) gene, and for AtuORF23 and AtuORF1, flanking the pat gene, both occurring on the *A. tumefaciens* plasmid pTi15955. Double homologous recombination of transgenic plant DNA with plasmid DNA of *A. tumefaciens* could result in an insertion of the *cry1Ac* (*synpro*) at the AtuORF23 site and an insertion of the *pat* gene into the region between AtuORF23 and AtuORF1.

A. tumefaciens occurs in soil, water and in the plant rhizosphere. It is therefore not expected to be prevalent in the main receiving environments, i.e. the gastrointestinal tract of humans or animals. However, occurrence of the recombinant genes outside of the immediate receiving environment (through faecal material) in soils cannot be ruled out, and is therefore also considered when assessing the risks associated with a HGT.

As indicated above, there is a theoretical possibility of double homologous recombination resulting in a plasmid with the codon-optimised *cry1Ac* (*synpro*) gene or *pat* gene, both under the control of a plant viral CsVMV promoter, in *A. tumefaciens*. Such recombination event, however, is expected to be

[34] Dossier: Part II – Section E3.1; 3.2.

expressed at low levels by the plant viral promoter. Compared to the natural bacterial variants of these genes, it is likely that the plant-optimised genes will result in less functional proteins in bacteria. The acquisition of the *cry1Ac* (*synpro*) and pat gene is unlikely to confer a selective advantage to *A. tumefaciens* in their natural habitats except when the *pat* gene changes the sensitivity to glufosinate ammonium when it is applied. The insertion of the *pat* gene on the Ti plasmid would result in a dysfunctional plasmid due to the loss of a large fragment between AtuORF23 and AtuORF1. The theoretical potential of recombinant Ti-plasmids to transfer the recombinant genes from *A. tumefaciens* back to infected plants would be limited to the infected tissue (crown gall tumour) and not enter the germline. For the *pat* gene insertion the Ti plasmid would lose its potential for crown gall formation, due to the loss of plasmid encoded genes between AtuORF23 and AtuORF1.

In addition to homology-based recombination processes, non-homologous (illegitimate) recombination that does not require 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, 2009). Thus, this process, in comparison with homologous recombination, is not considered to contribute significantly to HGT events.

In summary, the GMO Panel identified two theoretical scenarios where recombinant genes could be transferred by double homologous recombination onto a plasmid of a soil bacterium. The transfer would, however, not provide an immediate selective advantage to the recipient bacteria, except for glufosinate ammonium. Considering the occurrence of natural variants of *cry* and *pat* genes in environmental bacterial communities, such recombinant genes would not introduce new properties to the environment. Therefore, the GMO Panel did not identify a concern in relation to the theoretically possible horizontal transfer of recombinant genes from DAS-81419-2 to bacteria.

2) Plant-to-plant gene transfer

Considering the scope of the application EFSA-GMO-NL-2013-116 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, while the cultivated soybean, *G. max*, and its wild and semiwild 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). Since *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 since they have not been reported in other parts of the world where the cultivated soybean is grown (Dorokhov et al., 2004; Lu, 2005), the plant-to-plant gene transfer from soybean is restricted to cultivated areas and 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-81419-2 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-81419-2 seeds are processed in the countries of production or in ports of importation. The overall likelihood of cross-pollination between occasional 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 and target organisms[35]

Considering the scope of the application EFSA-GMO-NL-2013-116, potential interactions of occasional feral soybean DAS-81419-2 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[36]

Considering that environmental exposure of non-target organisms to stored GM seeds, spilled GM seeds or GM plants arising from spilled GM seeds is limited, potential exposure of non-target organisms sensitive to Cry1Ac and/or Cry1F proteins is likely to be very low and of no relevance.

The GMO Panel evaluated whether the expressed Cry1Ac and Cry1F proteins might potentially affect non-target organisms by entering the environment through faecal material of animals fed GM soybean. Cry proteins are degraded by enzymatic activity in the gastrointestinal tract, meaning that only low amounts of intact Cry proteins would remain in the faeces. This was demonstrated for Cry1Ab (Einspanier et al., 2004; Lutz et al., 2005, 2006; Wiedemann et al., 2006; Guertler et al., 2008; Paul et al., 2010). Further degradation of the protein in the manure and faeces will take place because of microbiological proteolytic activity. In addition, there will be further degradation of Cry proteins in soil, reducing the possibility for exposure of potentially sensitive non-target organisms. Although Cry proteins may bind to clay minerals and organic substances in soil, thereby reducing their availability to microorganisms for degradation, there are no indications of persistence and accumulation of Cry proteins from GM crops in soil (Gruber et al., 2011; Valldor et al., 2015). The GMO Panel is not aware of evidence of released Cry proteins from GM plants causing significant negative effects on soil microorganisms.

Considering the scope of the application, it can be concluded that the exposure of potentially sensitive non-target organisms to the Cry1Ac and Cry1F proteins is likely to be very low and of no biological relevance, regardless of potential synergistic interactions that might occur between the different Cry proteins.

Interactions with the abiotic environment and on biogeochemical cycles[37]

Considering the scope of the application EFSA-GMO-NL-2013-116, and the low level of exposure to the environment, potential interactions of occasional feral soybean DAS-81419-2 plants arising from seed import spills with the abiotic environment and biogeochemical cycles are not considered a relevant issue by the GMO Panel.

3.4.2. Post-market environmental monitoring[38]

The objectives of a PMEM plan, according to Annex VII of Directive 2001/18/EC are to: (1) 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) 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 includes: (1) the description of a monitoring approach involving operators (federations involved in soybean import and processing), reporting to the applicants, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment; (2) a coordinating system established by EuropaBio for the collection of information recorded by the various operators (Lecoq et al., 2007; Windels et al., 2008); and (3) the use of networks of existing surveillance systems. 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 that the scope of the PMEM plan provided by the applicant is consistent with the scope of soybean DAS-81419-2. As the ERA does not cover cultivation and did not identify potential adverse environmental effects from

[35] Dossier: Part II – Section E3.3.
[36] Dossier: Part II – Section E3.4.
[37] Dossier: Part II – Section E3.6.
[38] Dossier: Part II – Section E4; additional information: 27/8/2014.

soybean DAS-81419-2, no case-specific monitoring is necessary. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

3.4.3. Conclusion on the environmental risk assessment and monitoring plan

In the case of accidental release into the environment of viable seeds of soybean DAS-81419-2, there are no indications of an increased likelihood of establishment and spread of occasional feral soybean DAS-81419-2 plants, unless these plants are exposed to glufosinate ammonium-based herbicides or infested by insect pests that are susceptible to the Cry1F and Cry1Ac proteins. 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-2013-116, interactions of soybean DAS-81419-2 with the biotic and abiotic environment are not considered to be relevant issues. Bioinformatic analysis of the inserted DNA identified sufficient sequence identity with bacterial DNA which could theoretically facilitate the transfer of a plant codon-optimised *cry* gene and a *pat* gene onto a plasmid of a soil bacterium. Considering the occurrence of natural variants of such genes in environmental bacterial communities, the GMO panel did not identify a concern in relation to the theoretically possible HGT to bacteria.

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-81419-2 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-81419-2 and the GMO Panel guidelines on the PMEM of GM plants (EFSA GMO Panel, 2011b).

4. Conclusions

The EFSA GMO Panel was asked to carry out a scientific assessment of soybean DAS-81419-2 for import, processing, and food/feed uses in accordance with Regulation (EC) No 1829/2003. The molecular characterisation data provided for soybean DAS-81419-2 did not give rise to safety issues. The GMO Panel concluded that none of the differences identified in the compositional, agronomic and phenotypic characteristics of soybean DAS-81419-2 required further assessment regarding food and feed safety. No concerns regarding the potential toxicity or allergenicity of the newly expressed Cry1F, Cry1Ac and the PAT proteins were identified, and no evidence that the genetic modification might significantly change the overall allergenicity of soybean DAS-81419-2 was found. The nutritional value of food and feed derived from soybean DAS-81419-2 is not expected to differ from that of food and feed derived from non-GM soybean varieties. The GMO Panel concludes that soybean DAS-81419-2, assessed in this application, is as safe and nutritious as its conventional counterpart and the non-GM soybean reference varieties tested. 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-81419-2 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, HGT from soybean DAS-81419-2 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-81419-2.

In conclusion, the GMO Panel considers that the information available for soybean DAS-81419-2 addresses the scientific comments raised by the Member States and that the soybean DAS-81419-2, as described in this application, is as safe and nutritious as its conventional counterpart and non-GM reference varieties tested with respect to potential effects on human and animal health and the environment in the context of the scope of the application.

Documentation provided to EFSA

1) Letter from the Competent Authority of the Netherlands, received on 13 May 2013, concerning a request for placing on the market of soybean DAS-81419-2 submitted in accordance with Regulation (EC) No 1829/2003 by Dow AgroSciences (EFSA-GMO-NL-2013-116).
2) Acknowledgement letter dated 7 June 2103 from EFSA to the Competent Authority of the Netherlands.

3) Letter from EFSA to applicant dated 25 June 2013 requesting additional information under completeness check.
4) Letter from applicant to EFSA received on 21 November 2013 providing additional information under completeness check.
5) Letter from EFSA to applicant dated 12 December 2013 requesting additional information under completeness check.
6) Letter from applicant to EFSA received on 17 January 2014 providing additional information under completeness check.
7) Letter from EFSA to applicant dated 7 February 2014 delivering the 'Statement of Validity' for application EFSA-GMO-NL-2013-116, soybean DAS-81419-2 submitted by Dow AgroSciences under Regulation (EC) No 1829/2003.
8) Letter from EFSA to applicant dated 12 February 2014 requesting additional information and stopping the clock on behalf of the EURL-GMFF.
9) Letter from EFSA to applicant dated 21 March 2014 re-starting the clock on behalf of the EURL-GMFF.
10) Letter from EFSA to applicant dated 8 April 2014 requesting additional information and stopping the clock.
11) Letter from EFSA to applicant dated 28 May 2014 requesting additional information and maintaining the clock stopped.
12) Letter from applicant to EFSA received on 18 June 2014 providing additional information.
13) Letter from EFSA to applicant dated 23 June 2014 requesting additional information and maintaining the clock stopped.
14) Letter from applicant to EFSA received on 27 August 2014 providing additional information.
15) Letter from EFSA to applicant dated 9 September 2014 requesting additional information and maintaining the clock stopped.
16) Letter from applicant to EFSA received on 12 November 2014 providing additional information.
17) Letter from applicant to EFSA received on 25 November 2014 providing additional information.
18) Letter from applicant to EFSA received on 16 December 2014 providing additional information spontaneously.
19) Letter from EFSA to applicant dated 1 October 2015 requesting additional information and maintaining the clock stopped.
20) Letter from EFSA to applicant dated 9 November 2015 annulling the question sent on 1 October 2015.
21) Letter from EFSA to applicant dated 23 November 2015 requesting additional information and maintaining the clock stopped.
22) Letter from applicant to EFSA received on 24 November 2015 providing additional information.
23) Letter from applicant to EFSA received on 18 January 2016 providing additional information.
24) Letter from applicant to EFSA received on 22 January 2016 providing clarifications on the scope of the application.
25) Letter from EFSA to applicant dated 11 February 2016 re-starting the clock.
26) Letter from EFSA to applicant dated 17 February 2016 requesting additional information and stopping the clock.
27) Letter from applicant to EFSA received on 7 March 2016 providing additional information.
28) Email from EFSA to applicant dated 10 March 2016 re-starting the clock from 7 March 2016.
29) Letter from EFSA to applicant dated 15 March 2016 requesting additional information and stopping the clock.
30) Letter from applicant to EFSA received on 29 March 2016 providing additional information.
31) Letter from applicant to EFSA received on 31 March 2016 providing additional information (sequence information) spontaneously.
32) Letter from EFSA to applicant dated 25 April 2016 requesting additional information and stopping the clock on behalf of the EURL-GMFF.
33) Letter from EFSA to applicant dated 26 May 2016 requesting additional information and maintaining the clock stopped.
34) Letter from EFSA to applicant dated 10 June 2016 requesting additional information and maintaining the clock stopped.

35) Letter from applicant to EFSA received on 13 June 2016 providing additional information.
36) Letter from applicant to EFSA received on 17 June 2016 providing additional information.
37) Letter from EFSA to applicant dated 10 August 2016 requesting additional information and maintaining the clock stopped.
38) Letter from applicant to EFSA received on 1 September 2016 providing additional information.
39) Letter from EURL to EFSA dated 7 September 2016 requesting to re-start the clock.
40) Letter from EFSA to applicant dated 8 September 2016 re-starting the clock.

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, Júnior 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.

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

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.

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

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.

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), 2009. 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 GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010a. 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), 2010b. 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), 2010c. Scientific Opinion on application (EFSA-GMO-NL-2005-16) for the placing on the market of insect resistant genetically modified cotton (Gossypium hirsutum L.) 281-24-236 × 3006-210-23 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Dow AgroSciences. EFSA Journal 2010;8(6):1644, 32 pp. doi:10.2903/j.efsa.2010.1644

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010d. EFSA panel on genetically modified organisms (GMO). 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), 2011a. 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

Einspanier R, Lutz B, Rief S, Berezina O, Zverlov V, Schwarz W and Mayer J, 2004. Tracing residual recombinant feed molecules during digestion and rumen bacterial diversity in cattle fed transgenic maize. European Food Research and Technology, 218, 269–273.

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.

Gruber H, Paul V, Meyer HDH and Müller M, 2011. Determination of insecticidal Cry1Ab protein in soil collected in the final growing seasons of a nine-year field trial of Bt maize MON810. Transgenic Research, 21, 77–88.

Guertler P, Lutz B, Kuehn R, Meyer HHD, Einspanier R, Killermann B and Albrecht C, 2008. Fate of recombinant DNA and Cry1Ab protein after ingestion and dispersal of genetically modified maize in comparison to rapeseed by fallow deer (*Dama dama*). European Journal of Wildlife Research, 54, 36–43.

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.

Hammond B, Kough J, Herouet-Guicheney C and Jez JM on behalf of the ILSI International Food Biotechnology Committee Task Force on the Use of Mammalian Toxicology Studies in the Safety Assessment of GM Foods, 2013. Toxicological evaluation of proteins introduced into food crops. Critical Reviews in Toxicology, 43: Suppl. 2, 25–42.

Hülter N and Wackernagel W, 2008. Double illegitimate recombination events integrate DNA segments through two different mechanisms during natural transformation of *Acinetobacter baylyi*. Molecular Microbiology, 67, 984–995.

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(S2), 112.

Koch MS, Ward JM, Levine SL, Baum JA, Vicini JL and Hammond BG, 2015. The food and environmental safety of Bt crops. Frontiers in Plant Science, 6, 283.

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(S1), 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.

Lu BR, 2005. Multidirectional gene flow among wild, weedy, and cultivated soybeans. In: Gressel J (ed.). *Crop Ferality and Volunteerism*, OECD + Taylor & Francis, Boca Raton, FL. pp. 137–147.

Lutz B, Wiedemann S, Einspanier R, Mayer J and Albrecht C, 2005. Degradation of Cry1Ab protein from genetically modified maize in the bovine gastrointestinal tract. Journal of Agricultural and Food Chemistry, 53, 1453–1456.

Lutz B, Wiedemann S and Albrecht C, 2006. Degradation of transgenic Cry1Ab DNA and protein in Bt-176 maize during the ensiling process. Journal of Animal Physiology and Animal Nutrition (Berlin), 90, 116–123.

Monier J-M, Bernillon D, Kay E, Faugier A, Rybalka O, Dessaux Y, Simonet P and Vogel TM, 2007. Detection of potential transgenic plant DNA recipients among soil bacteria. Environmental Biosafety Research, 6, 71–83.

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.

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. ENV/JM/MONO (2001)15.

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. ENV/JM/MONO(2012)24.

Overballe-Petersen S, Harms K, Orlando LA, Mayar JV, Rasmussen S, Dahl TW, Rosing MT, Poole AM, Sicheritz-Ponten T, Brunak S, Inselmann S, de Vries J, Wackernagel W, Pybus OG, Nielsen R, Johnsen PJ, Nielsen KM and Willerslev E, 2013. Bacterial natural transformation by highly fragmented and damaged DNA. Proceedings of the National Academy of Sciences of the USA, 110, 19860–19865.

Owen MDK, 2005. Maize and soybeans – Controllable volunteerism without ferality? In: Gressel J (ed.). *Crop Ferality and Volunteerism*, OECD + Taylor & Francis, Boca Raton, FL. pp. 149–165.

Paul V, Guertler P, Wiedemann S and Meyer HHD, 2010. Degradation of Cry1Ab protein from genetically modified maize (MON810) in relation to total dietary feed proteins in dairy cow digestion. Transgenic Research, 19, 683–689.

Ray JD, Kilen TC, Abel CA and Paris RL, 2003. Soybean natural cross-pollination rates under field conditions. Environmental Biosafety Research, 2, 133–138.

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.

Valldor P, Miethling-Graff R, Martens R and Tebbe CC, 2015. Fate of the insecticidal Cry1Ab protein of GM crops in two agricultural soils as revealed by 14C-tracer studies. Applied Microbiology and Biotechnology, 99, 7333–7341.

Vazquez RI, Moreno-Fierro L, Neri-Bazan L, De La Riva GA and Lopez-Revilla R, 1999. *Bacillus thuringiensis* Cry1Ac protoxin is a potent systemic and mucosal adjuvant. Scandinavian Journal of Immunology, 49, 578–584.

de Vries J and Wackernagel W, 2002. Integration of foreign DNA during natural transformation of Acinetobacter sp. by homology-facilitated illegitimate recombination. Proceedings of the National Academy of Sciences of the USA. 99, 2094–2099.

Wiedemann S, Lutz B, Kurtz H, Schwarz FJ and Albrecht C, 2006. *In situ* studies on the time-dependent degradation of recombinant corn DNA and protein in the bovine rumen. Journal of Animal Science, 84, 135–144.

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(S2), 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

AA	amino acid
ADF	acid detergent fibre
AtuORF	*Agrobacterium tumefaciens* open reading frame
bp	base pair
CsVMV	*Cassava vein mosaic virus*
ELISA	enzyme-linked immunosorbent assay
ERA	environmental risk assessment
EURL-JRC	European Union Reference Laboratories-Joint Research Centre
FA	fatty acid
GM	genetically modified
GMO	genetically modified organisms
GMO Panel	EFSA Panel on Genetically Modified Organisms
HGT	horizontal gene transfer
HR	homologous recombination
IgE	immunoglobulin E
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
PAT	phosphinothricin acetyltransferase
PCR	polymerase chain reaction
PMEM	post-market environmental monitoring
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
T-DNA	transfer-deoxyribonucleic acid
UTR	untranslated region
WSR	Wilcoxon Signed Rank