SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 15 October 2020

doi: 10.2903/j.efsa.2020.6302

Scientific Opinion on application EFSA-GMO-NL-2016-132 for authorisation of genetically modified of insect-resistant and herbicide-tolerant soybean DAS–81419–2 × DAS–44406–6 for food and feed uses, import and processing submitted in accordance with Regulation (EC) No 1829/2003 by Dow Agrosciences LCC

EFSA Panel on Genetically Modified Organisms (GMO),
Hanspeter Naegeli, Jean-Louis Bresson, Tamas Dalmay, Ian Crawford Dewhurst,
Michelle M Epstein, Leslie George Firbank, Philippe Guerche, Jan Hejatko,
Francisco Javier Moreno, Ewen Mullins, Fabien Nogué, Nils Rostoks,
Jose Juan Sánchez Serrano, Giovanni Savoini, Eve Veromann, Fabio Veronesi,
Fernando Álvarez, Michele Ardizzone, Giacomo De Sanctis, Yann Devos,
Antonio Fernandez Dumont, Silvia Federici, Andrea Gennaro, Jose Ángel Gómez Ruiz,
Anna Lanzoni, Franco Maria Neri, Nikoletta Papadopoulou, Konstantinos Paraskevopoulos and
Tommaso Raffaello

Abstract

Soybean DAS–8419–2 × DAS–44406–6 was developed to provide protection against certain lepidopteran pests and tolerance to 2,4–dichlorophenoxyacetic acid and other related phenoxy herbicides, and glyphosate- and glufosinate ammonium-containing herbicides. The Genetically Modified Organisms (GMO) Panel previously assessed the two single soybean events and did not identify safety concerns. No new data on the single soybean events, leading to modification of the original conclusions on their safety have been identified. The molecular characterisation, comparative analysis (agronomic, phenotypic and compositional characteristics) and the outcome of the toxicological, allergenicity and nutritional assessment indicate that the combination of the single soybean events and of the newly expressed proteins in the two-event stack soybean does not give rise to food and feed safety and nutritional concerns. In the case of accidental release of viable DAS–8419–2 × DAS–44406–6 seeds into the environment, soybean DAS–8419–2 × DAS–44406–6 would not raise environmental safety concerns. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of soybean DAS–8419–2 × DAS–44406–6. In conclusion, the GMO Panel considers that soybean DAS–8419–2 × DAS–44406–6, as described in this application, is as safe as its conventional counterpart and the non-genetically modified soybean reference varieties tested with respect to potential effects on human and animal health and the environment.

Keywords: AAD–12, Cry1Ac, Cry1F, PAT, 2mEPSPS, insect–resistance, herbicide–tolerance

Requestor: European Commission

Question number: EFSA-Q-2016-00195

Correspondence: GMO_secretariat_applications@efsa.europa.eu

Panel members: Hanspeter Naegeli, Jean-Louis Bresson, Tamas Dalmay, Ian Crawford Dewhurst, Michelle M Epstein, Leslie George Firbank, Philippe Guerche, Jan Hejatko, Francisco Javier Moreno, Ewen Mullins, Fabien Nogué, Nils Rostoks, Jose Juan Sánchez Serrano, Giovanni Savoini, Eve Veromann and Fabio Veronesi.

Acknowledgements: The Panel wishes to thank the members of the Working Groups on Molecular Characterisation, Food and Feed Safety Assessment and Working Group on Comparative Analysis and Environmental Risk Assessment for the preparatory work on this scientific output and EFSA staff members Irene Muñoz–Guajardo, Sonia Hernández Valero and Sylvie Mestdagh for the support provided to this scientific output.

Suggested citation: EFSA Panel on Genetically Modified Organisms (GMO), Naegeli H, Bresson J-L, Dalmay T, Dewhurst IC, Epstein MM, Firbank LG, Guerche P, Hejatko J, Moreno FJ, Mullins E, Nogué F, Rostoks N, Sánchez Serrano JJ, Savoini G, Veromann E, Veronesi F, Álvarez F, Ardizzone M, De Sanctis G, Devos Y, Dumont AF, Federici S, Gennaro A, Gómez Ruiz JÁ, Lanzoni A, Neri FM, Papadopoulou N, Paraskevopoulos K and Raffaello T, 2020. Scientific Opinion on application EFSA-GMO-NL-2016-132 for authorisation of genetically modified of insect-resistant and herbicide-tolerant soybean DAS–81419–2 × DAS–44406–6 for food and feed uses, import and processing submitted in accordance with Regulation (EC) No 1829/2003 by Dow Agrosciences LCC. EFSA Journal 2020;18(11):6302, 37 pp. https://doi.org/10.2903/j.efsa.2020.6302

ISSN: 1831-4732

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

Summary

In this scientific opinion, the scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (hereafter referred to as the 'GMO Panel') reports on the outcome of its risk assessment of soybean DAS–81419–2 × DAS–44406–6 according to the scope as defined in the application EFSA–GMO–NL–2016-132. The GMO Panel conducted the assessment of soybean DAS–81419–2 × DAS–44406–6 in line with the principles described in Regulation (EU) No 503/2013 and its applicable guidelines for the risk assessment of genetically modified (GM) plants.

The two-event stack soybean was produced by conventional crossing to combine two single soybean events: DAS-81419-2 (expressing the proteins Cry1F, Cry1Ac and PAT) and DAS–44406–6 (expressing the proteins 2mEPSPS, AAD–12 and PAT) to confer protection against specific lepidopteran pests and tolerance to glufosinate ammonium-, glyphosate-containing herbicides and 2,4-dichlorophenoxyacetic acid and other related phenoxy herbicides.

The single events DAS–81419–2 and DAS–44406–6 were previously assessed by the GMO Panel and no concerns on their safety were identified. No new safety issue was identified by updated bioinformatic analyses, nor reported by the applicant concerning the two single soybean events, since the publication of the respective scientific opinions. Consequently, the GMO Panel considers that its previous conclusions on the safety of the single soybean events remain valid.

For the two-event stack soybean, the risk assessment included the molecular characterisation of the inserted DNA and analysis of protein expression. An evaluation of the comparative analysis of agronomic/phenotypic and compositional characteristics was undertaken, and the safety of the newly expressed proteins and the whole food and feed were evaluated with respect to potential toxicity, allergenicity and nutritional characteristics. An evaluation of environmental impacts and the post-market environmental monitoring (PMEM) plan was also carried out.

The molecular characterisation data establish that the events stacked in the two-event stack soybean have retained their integrity. Protein expression analyses show that the levels of the newly expressed proteins are comparable in the two-event stack and in the single events. No indications of additional interactions that may affect the integrity of the events and the levels of the newly expressed proteins in this two-event stack soybean were identified.

The comparative analysis of forage and seed composition and agronomic and phenotypic characteristics identified no differences between the two-event stack soybean and the conventional counterpart that required further assessment for food and feed safety or environmental impact, except for 100–seed weight and for the levels of acid detergent fibre (ADF), neutral detergent fibre, crude fibre and phosphorus in forage and ADF, phosphatidylinositol, glutamic acid and lectin activity in seeds. Those changes were further assessed and not found to have a safety impact.

The molecular characterisation, the comparative analysis and the outcome of the toxicological and allergenicity assessment indicate that the combination of the single soybean events and of the newly expressed proteins in the two-event stack soybean does not give rise to food and feed safety and nutritional concerns. The GMO Panel concludes that soybean DAS–81419–2 × DAS–44406–6, as described in this application, is as safe as and nutritionally equivalent to the conventional counterpart and the commercial non-GM soybean reference varieties tested.

Considering the combined events and their potential interactions, the outcome of the comparative analysis and the routes and levels of exposure, the GMO Panel concludes that soybean DAS–81419–2 × DAS–44406–6 would not raise safety concerns in the case of accidental release of viable GM soybean seeds into the environment.

Given the absence of safety concerns for foods and feeds from the two-event stack soybean, the GMO Panel considers that post-market monitoring of food and feed is not necessary. The PMEM plan and reporting intervals are in line with the intended uses of the two-event stack soybean.

Based on the relevant publications identified through the literature searches, the GMO Panel does not identify any safety issues pertaining to the uses of soybean DAS–81419–2 × DAS–44406–6. In the context of annual PMEM reports, the applicant could further fine-tune future literature searches according to the GMO Panel recommendations.

The GMO Panel concludes that soybean DAS–81419–2 × DAS–44406–6, as described in this application, is as safe as the conventional counterpart and the tested non-GM reference varieties with respect to potential effects on human and animal health and the environment.

Table of contents

1. Introduction

The scope of application EFSA-GMO-NL-2016-132 is for food and feed uses, import and processing of the genetically modified (GM) herbicide-tolerant and insect-resistant soybean DAS–81419–2 × DAS–44406–6 in the European Union (EU).

1.1. Background

On 2 March 2016, the European Food Safety Authority (EFSA) received from the Competent Authority of The Netherlands application EFSA-GMO-NL-2016–132 for authorisation of soybean DAS–81419–2 × DAS–44406–6 (Unique Identifier DAS-81419-2 × DAS-444Ø6-6), submitted by Dow AgroSciences LLC (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003[1].

Following receipt of application EFSA–GMO–NL–2016–132, EFSA informed EU Member States and the European Commission, and made the application available to them. Simultaneously, EFSA published the summary of the application.[2]

EFSA checked the application for compliance with the relevant requirements of EFSA guidance documents, and, when needed, asked the applicant to supplement the initial application. On 9 August 2016, EFSA declared the application valid.

From the date of validity, EFSA and its scientific Panel on Genetically Modified Organisms (hereafter referred to as 'the GMO Panel') endeavoured to respect a time limit of six months to issue a scientific opinion on application EFSA–GMO–NL–2016–132. Such time limit was extended whenever EFSA and/or its GMO Panel requested supplementary information to the applicant. According to Regulation (EC) No 1829/2003, any supplementary information provided by the applicant during the risk assessment was made available to the EU Member States and European Commission (for further details, see the section 'Documentation', below).

In accordance with Regulation (EC) No 1829/2003, EFSA consulted the nominated risk assessment bodies of EU Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC[3]. The EU Member States had three months to make their opinion known on application EFSA–GMO–NL–2016–132 as of date of validity.

1.2. Terms of Reference as provided by the requestor

According to Articles 6 and 18 of Regulation (EC) No 1829/2003, EFSA and its GMO Panel were requested to carry out a scientific risk assessment of soybean DAS–81419–2 × DAS–44406–6 in the context of its scope as defined in application EFSA-GMO-NL-2016-132.

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).

The relevant information is made available in the EFSA Register of Questions including the information required under Annex II to the Cartagena Protocol; a labelling proposal, a post-market environmental monitoring (PMEM) plan as provided by the applicant, the method(s), validated by the Community reference laboratory, for detection, including sampling, identification of the transformation event in the food-feed and/or foods-feeds produced from it, and the appropriate reference materials.[4]

2. Data and methodologies

2.1. Data

The GMO Panel based its scientific risk assessment of soybean DAS–81419–2 × DAS–44406–6 on the valid application EFSA–GMO-NL–2016–132, additional information provided by the applicant during the risk assessment, relevant scientific comments submitted by the Member States and relevant peer-reviewed scientific publications. In addition to this comprehensive information package, the GMO Panel also received unpublished studies submitted by the applicant in order to comply with the specific

[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.
[2] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2016-00195
[3] 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.
[4] *[Cross-refer to footnote number 2]*.

provisions of Regulation (EU) No 503/2013[5]. A list of these additional unpublished studies is provided in Appendix A.

2.2. Methodologies

The GMO Panel conducted its assessment in line with the principles described in Regulation (EU) No 503/2013, its applicable guidelines (EFSA GMO Panel, 2010a, 2011a,b, 2015, 2017a) and explanatory notes and statements (EFSA, 2017a,b, 2019a,b) for the risk assessment of GM plants.

For the assessment of 90-day animal feeding studies, the GMO Panel took into account the criteria included in the EFSA Scientific Committee guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food and feed (EFSA Scientific Committee, 2011) and the explanatory statement for its applicability (EFSA, 2014).

The GMO Panel also assessed the applicant's literature searches, which include a scoping review, following the recommendations on literature searching outlined in EFSA (2010, 2017a). In the context of the contracts OC/EFSA/GMO/2013/01 and OC/EFSA/GMO/2014/01, contractors performed preparatory work and delivered reports on the methods applied by the applicant in performing bioinformatic and statistical analyses, respectively.

3. Assessment

3.1. Introduction

Application EFSA–GMO–NL–2016–132 covers the two-event stack soybean DAS–81419–2 × DAS–44406–6 produced by conventional crossing of events DAS–81419–2 and DAS–44406–6. The scope of this application is for food and feed uses, import and processing, but excludes cultivation within the EU.

Soybean DAS-81419-2 × DAS-44406-6 was developed to provide protection against certain lepidopteran pests and tolerance to glyphosate-containing herbicides, 2,4-dichlorophenoxyacetic acid (2,4-D) and other related phenoxy herbicides and glufosinate ammonium-containing herbicides. Protection against lepidopteran pests and tolerance to these herbicides is achieved by the expression of the Cry1F and Cry1Ac, 5-enolpyruvyl-shikimate-3-phosphate synthase (2mEPSPS), aryloxyalkanoate dioxygenase (AAD–12) and phosphinothricin acetyltransferase (PAT) proteins.

The two single soybean events DAS-81419-2 and DAS-44406-6 have been previously assessed by the GMO Panel (Table 1) and no concerns for human and animal health or environmental safety were identified.

Table 1: Single soybean events assessed by the GMO Panel

Event	Application	EFSA Scientific Opinion
DAS-81419-2	EFSA-GMO-NL-2013-116	EFSA GMO Panel (2016)
DAS-44406-6	EFSA-GMO-NL-2013-106	EFSA GMO Panel (2017b)

3.2. Updated information on the events[6]

Since the publication of the respective GMO Panel Scientific Opinions (Table 1), no safety issues concerning the two single soybean events have been reported by the applicant.

Updated bioinformatic analyses for soybean events DAS-81419-2 and DAS–44406–6 confirm that no known endogenous genes were disrupted by any of the inserts.

Updated bioinformatic analyses of the amino acid sequences of the newly expressed proteins Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS confirm previous results indicating no significant similarities to toxins and allergens. In addition, updated bioinformatics analyses of the newly created Open Reading Frames (ORFs) within the inserts or spanning the junctions between the insert and the flanking regions for events DAS-81419-2 and DAS–44406–6, confirm previous results that do not indicate significant similarities to toxins or allergens for any of the events in soybean DAS–81419–2 × DAS–44406–6 (Table 1).

In order to assess the possibility for horizontal gene transfer (HGT) by homologous recombination, the applicant performed a sequence identity analysis for events DAS-81419-2 and DAS–44406–6 to

[5] Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006 Text with EEA relevance OJ L 157, 8.6.2013, p. 1–48.

[6] Dossier: Part II – Section 1.2.2.2; additional information: 15/5/2017, 10/5/2019 and 14/5/2020.

microbial DNA. The likelihood and potential consequences of plant-to-bacteria gene transfer are described in Section 3.7.2.1.

Based on the above information, the GMO Panel considers that its previous conclusions on the safety of the single soybean events remain valid.

3.3. Systematic literature review[7]

The GMO Panel assessed the applicant's literature searches on soybean DAS–81419–2 × DAS–44406–6, which included a scoping review, according to the guidelines given in EFSA (2010, 2017a). A systematic review as referred to in Regulation (EU) No 503/2013 has not been provided in support to the risk assessment of application EFSA–GMO–NL–2016–132. Based on the outcome of the scoping review, the GMO Panel agrees that there is limited value of undertaking a systematic review for soybean DAS–81419–2 × DAS–44406–6 at present.

Although the overall quality of the performed literature searches is acceptable, the GMO Panel considers that future searches on soybean DAS–81419–2 × DAS–44406–6 could be fine-tuned further. The GMO Panel therefore recommends the applicant to ensure that enough search term variation is used in the free–text (covering possible synonyms, related terms, acronyms, spelling variants, old and new terminology, brand and generic names, lay and scientific terminology, common typos, translation issues) and the subject-indexing terms.

Based on the relevant publications identified through the literature searches (Appendix B), the GMO Panel does not identify any safety issues pertaining to the intended uses of soybean DAS–81419–2 × DAS–44406–6.

3.4. Molecular characterisation[8]

In line with the requirements laid down by Regulation (EU) No 503/2013, the possible impact of the combination of the events on their integrity, the expression levels of the newly expressed proteins or the biological functions conferred by the individual inserts are considered below.

3.4.1. Genetic elements and their biological function

The two-event stack soybean was obtained by conventional crossing of soybean events DAS–81419–2 and DAS–44406–6. The structure of the inserts introduced into soybean events DAS–81419–2 and DAS–44406–6 is described in detail in the respective GMO Panel Scientific Opinions (Table 1) and no new genetic modifications were involved. Genetic elements in the expression cassettes of the single events are summarised in Table 2. Intended effects of the inserts in the two-event stack soybean are summarised in Table 3.

Based on the known biological function of the newly expressed proteins (Table 3), the only potential functional interactions at the biological level are between the two Cry proteins in susceptible insects. This potential for functional interactions was already present in the single event DAS–81419–2.

Table 2: Genetic elements in the expression cassettes of the events stacked in the two-event stack soybean DAS–81419–2 × DAS–44406–6

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
DAS-81419-2	*AtUbi10* (*Arabidopsis thaliana*)*	*AtUbi10* (*A. thaliana*)	No	*cry1F* (*Bacillus thuringiensis*)	*AtuORF23 3′ UTR* (*Agrobacterium tumefaciens*)
	CsVMV (*Cassava Vein Mosaic Virus*)	*CsVMV* (*Cassava Vein Mosaic Virus*)	No	*cry1Ac (synpro)* (*B. thuringiensis*)	*AtuORF23 3′ UTR* (*A. tumefaciens*)
	CsVMV (*Cassava Vein Mosaic Virus*)	*CsVMV* (*Cassava Vein Mosaic Virus*)	No	*pat* (*Streptomyces viridochromogenes*)	*AtuORF1 3′ UTR* (*A. tumefaciens*)

[7] Additional information: 15/5/2017, 13/12/2017 and 21/5/2019.
[8] Dossier: Part II – Sections 1.2.2.1, 1.2.2.2 and 1.2.2.3; additional information: 23/6/2017, 25/8/2017 and 14/5/2018.

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
DAS-44406-6	*H4A748* (*A. thaliana*)	*H4A748* (*A. thaliana*)	TPotp C (*Zea mays*)	*2mepsps* (*Z. mays*)	*H4A748 3′UTR* (*A. thaliana*)
	AtUbi10 (*A. thaliana*)	*AtUbi10* (*A. thaliana*)	No	*aad-12*† (*Delftia acidovorans*)	*AtuORF23 3′ UTR* (*A. tumefaciens*)
	CsVMV (*Cassava Vein Mosaic Virus*)	*CsVMV* (*Cassava Vein Mosaic Virus*)	No	*pat*† (*S. viridochromogenes*)	*AtuORF1 3′ UTR* (*A. tumefaciens*)

*: Source of genetic information.
†: Codon optimised.

Table 3: Characteristics and intended effects of the events stacked in the two-event stack soybean DAS–81419–2 × DAS–44406–6

Event	Protein	Donor organism and biological function	Intended effects in GM plant
DAS–81419–2	Cry1F	Based on genes from *Bacillus thuringiensis (Bt)*. *Bt* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (cry) genes (Schnepf et al., 1998)	Event DAS-81419-2 expresses a synthetic *cry1F* gene, consisting of parts of the *cry1Fa2* and *cry1Ca3* genes from *Bt* subsp. *aizawai* strain PS811 and part of the *cry1Ab1* gene from *Bt* subsp. *berliner* strain 1715. Cry1F is a protein toxic to certain lepidopteran larvae. (Cardineau et al., 2001; Gao et al., 2006)
	Cry1Ac (synpro)	Based on genes from *Bt*. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of *cry* genes (Schnepf et al., 1998)	Event DAS-81419-2 expresses a synthetic *cry1Ac* gene, consisting of parts of the following genes: *cry1Ac1* gene from *Bt* supsp. *kurstaki* strain HD73, *cry1Ca3* gene from *Bt* subsp. *aizawai* strain PS811, and *cry1Ab1* from *Bt* subsp. *berliner* strain 1715. Cry1Ac (synpro) is a protein toxic to certain lepidopteran larvae. (Cardineau et al., 2001; Gao et al., 2006)
	PAT	Based on a gene from *Streptomyces viridochromogenes* Tü494. Phosphinothricin-acetyl-transferase (PAT) enzyme acetylates L-glufosinate-ammonium (Thompson et al., 1987; Wohlleben et al., 1988; Eckes et al., 1989)	Event DAS-81419-2 expresses a synthetic *pat* gene. PAT acetylates L-glufosinate-ammonium and thereby confers tolerance to glufosinate ammonium-based herbicides
DAS-44406-6	2mEPSPS	Based on a gene from *Zea mays*. 5-enopyruvyl-shikimate-3-phosphate synthase (EPSPS) is an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Herrmann, 1995)	Event DAS-44406-6 expresses a 2mEPSPS protein which is a modified version of the endogenous EPSPS enzyme that confers tolerance to glyphosate-based herbicides (Lebrun et al., 2003)
	AAD-12	Based on a gene from *Delftia acidovorans*. Aryloxyalkanoate dioxygenases (AAD) cleave a range of xenobiotic herbicides, including 2,4-D (Wright et al., 2010)	Event DAS-44406-6 expresses *aad–12*, which is a synthetic, condon-optimised version of the *aad* gene. Expression of AAD-12 confers tolerance to 2,4–D
	PAT	Based on a gene from *Streptomyces viridochromogenes* Tü494. Phosphinothricin-acetyl-transferase (PAT) enzyme acetylates L-glufosinate-ammonium (Thompson et al., 1987; Wohlleben et al., 1988; Eckes et al., 1989)	Event DAS-44406-6 expresses a synthetic *pat* gene. PAT acetylates L–glufosinate-ammonium and thereby confers tolerance to glufosinate ammonium-based herbicides

3.4.2. Integrity of the events in the two-event stack soybean

The genetic stability of the inserted DNA over multiple generations in the single soybean events DAS–81419-2 and DAS–44406-6 was demonstrated previously (Table 1). Integrity of these events in soybean DAS–81419-2 × DAS–44406-6 was demonstrated by Sanger sequence analysis of polymerase chain reaction (PCR) products which showed that the sequences of the events in the two-event stack soybean are identical to the sequences originally reported for the two single events.

3.4.3. Information on the expression of the inserts

Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS protein levels were analysed by enzyme-linked immunosorbent assay (ELISA) in material harvested from field trials at nine locations in the USA in the 2012 growing season. Samples analysed included leaf (V5 and V10-12), forage (R3), root (R3) and seed (R8) from plants treated and not treated with 2,4-D, glufosinate and glyphosate. In order to assess the changes in protein expression levels which may result from potential interactions between the events, protein levels were determined for the two-event stack and the corresponding single events in different parts of the plant.

The levels of all the newly expressed proteins in the two-event stack soybean were comparable to those of the single events (Appendix C). Differences in PAT protein levels were expected because of the combination of soybean events DAS-81419-2 and DAS-44406-6 both producing PAT in the two-event stack soybean. The variability in protein expression data observed for Cry1Ac, Cry1F and 2mEPSPS proteins in some of the analysed tissues is discussed in Section 3.6.5. Based on the available data, there is no indication of an interaction that may affect the levels of the newly expressed proteins in this two-event stack soybean.

3.4.4. Conclusions of the molecular characterisation

The molecular data establish that the events stacked in soybean DAS-81419-2 × DAS-44406-6 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins are comparable in the two-event stack and in the single events. As regards the PAT protein, the results showed the expected higher levels of PAT in the two-event stack soybean compared to the single soybean events DAS-81419-2 and DAS-44406-6. Therefore, there is no indication of interaction that may affect the integrity of the events and the levels of the newly expressed proteins in this two-event stack soybean.

Based on the known biological function of the newly expressed proteins, there are no additional potential interactions of the proteins expressed in the stack compared to those in the single events.

3.5. Comparative analysis[9]

3.5.1. Overview of studies conducted for the comparative analysis

Application EFSA–GMO–NL–2016–132 presents data on agronomic and phenotypic characteristics, as well as on forage and seed composition of soybean DAS–81419–2 × DAS–44406–6 (Table 4).

Table 4: Overview of the comparative analysis studies to characterise the two-event stack GM soybean DAS–81419–2 × DAS–44406–6 provided in the application EFSA–GMO–NL–2016–132

Study focus	Study details	Comparator	Commercial non-GM reference varieties
Agronomic and phenotypic analysis	Field study, USA, 2012, nine sites(a)	Maverick	Six(b)
Compositional analysis			

(a): The field trials were located in Richland, IA; Atlantic, IA; Carlyle, IL; Wyoming, IL; Sheridan, IN; Kirksville, MO; Fisk, MO; York, NE; and Germansville, PA. A site located in Brunswick, NE, was excluded from the field trials due to a frost event (additional information: 28/2/2018).

(b): The commercial non-GM soybean reference varieties used in the 2012 field trials were Dyna-Gro V388SCN, Dyna-Gro 3410SCN, DSR 3510, Pioneer 93Y41, L&M 34, and Stine 3900-2.

[9] Dossier: Part II – Section 1.3; additional information: 25/8/2017, 28/2/2018, 23/3/2018, 14/5/2018, 27/8/2018, 21/9/2018, 4/12/2018, 18/1/2019, 11/2/2019, 2/5/2019, 4/11/2019 and 14/1/2020.

3.5.2. Experimental field trial design and statistical analysis

At each field trial site, the following materials were grown: soybean DAS–81419–2 × DAS–44406–6, the comparator Maverick and three commercial non-GM soybean reference varieties, all treated with conventional herbicides management regimes, and soybean DAS-81419-2 × DAS-44406-6 exposed to the intended glyphosate-, glufosinate-ammonium- and 2,4-D containing herbicides, in addition to the conventional herbicides.

The agronomic, phenotypic and compositional data were analysed as specified by the GMO Panel (EFSA GMO Panel, 2010b, 2011a). This includes, for each of the two treatments of soybean DAS–81419–2 × DAS–44406–6, the application of a difference test (between the GM soybean and its conventional counterpart) and an equivalence test (between the GM soybean and the set of commercial non-GM soybean reference varieties). The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).[10]

3.5.3. Suitability of selected test materials

3.5.3.1. Selection of the GM soybean line and comparator

The two single events DAS–81419–2 and DAS–44406–6 were developed via *Agrobacterium*-mediated transformation of the soybean cv. Maverick. The two events were combined in the Maverick genetic background by conventional crossing and stabilised. The soybean cv. Maverick was selected as the comparator in the studies conducted for the comparative analysis. The GMO Panel considers the selected comparator (Maverick) as the appropriate conventional counterpart for the comparative analysis.

The two-stack GM soybean DAS–81419–2 × DAS–44406–6 and Maverick are both in the same maturity group and appropriate for growing in a range of environments across North America.

Six commercial non-GM reference varieties with a maturity group ranging from III to IV were selected by the applicant. At each selected site, three reference varieties were tested (Table 4). On the basis of the provided information on maturity group classes, the GMO Panel considers the selected non-GM reference varieties as appropriate for the comparative assessment.

3.5.3.2. Seed production and quality

Seeds of soybean DAS–81419–2 × DAS–44406–6 and its conventional counterpart used in the 2012 field trials were produced, harvested and stored under similar conditions. Their purity and identity were verified via event-specific PCR and ELISA method analyses, showing purity and identity levels above 99%.

Seed germination was tested under greenhouse conditions. A reduction was identified in the germination capacity for soybean DAS-81419-2 × DAS-44406-6 compared to its conventional counterpart. The result was considered not to affect the quality of the field trials due to the small magnitude of the difference.

The GMO Panel considers that the starting seed used as test material in the agronomic, phenotypic and compositional studies was of acceptable quality.

3.5.3.3. Conclusion on suitability

The GMO Panel is of the opinion that soybean DAS–81419–2 × DAS–44406–6, the conventional counterpart and the non-GM soybean reference varieties were properly selected and are of sufficient quality. Therefore, the test materials are considered suitable for the comparative analysis.

3.5.4. Representativeness of the receiving environments

3.5.4.1. Selection of field trial sites

The selected field trials were located in commercial soybean-growing regions of North America. The soil and climatic characteristics of the selected fields were diverse,[11] corresponding to optimal, near-optimal and suboptimal conditions for soybean cultivation (Sys et al., 1993). The GMO Panel considers

[10] 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).

[11] Soil types of the field trials were silty clay loam, clay loam and silt loam. Mean temperatures and sum of precipitations during the usual soybean-growing season ranged, respectively, from 16.5°C to 22.4°C and from 365 mm to 765 mm.

that the selected sites reflect commercial soybean-growing regions in which the test materials are likely to be grown.

3.5.4.2. Meteorological conditions

Maximum and minimum mean temperatures and sum of precipitations were provided monthly. No exceptional weather conditions were reported at any of the selected sites; therefore, the GMO Panel considers that the meteorological data set falls within the historical range of climatic conditions normally occurring at these sites.

3.5.4.3. Management practices

The field trial included plots containing soybean DAS-81419-2 × DAS-44406-6, plots with the conventional counterpart and plots with non-GM soybean reference varieties, managed according to local agricultural practices. In addition, the field trials included plots containing soybean DAS-81419–2 × DAS-44406-6 managed following the same agricultural practices, plus exposed to the intended herbicides (2,4-D, glufosinate and glyphosate). 2,4-D– and glyphosate-containing herbicides were tank–mixed and applied three times: at planting/pre-emergence, at vegetative phase V3 and at maturity, approximately at growth stage R2. Glufosinate-containing herbicides were applied two times, at vegetative phase V5 and at growth stage R1.

At some field trial sites, sowing occurred later than usual, resulting in a deviation from standard management practices. The additional information indicated that the shorter and/or shifted growing cycle does not alter the capability to conclude on the comparative assessment. Therefore, the GMO Panel considers that the management practices including sowing, harvesting and application of plant protection products were acceptable.

3.5.4.4. Conclusions on representativeness

The GMO Panel concludes that the geographical locations, soil and climatic characteristics, meteorological conditions of the field trial sites and most of the management practices applied are typical of the receiving environments where the tested materials could be grown.

3.5.5. Agronomic and phenotypic analysis

Thirteen agronomic and phenotypic endpoints[12] were collected from the field trials (Table 4).

Four endpoints (seedling vigour, disease incidence, insect damage and pod shattering) did not fulfil the assumptions for parametric testing and were analysed with the Wilcoxon signed rank test. No significant differences were found between the GM soybean and the conventional counterpart for any of these endpoints.

The test of difference and the test of equivalence were applied to nine endpoints, with the following results:

- For soybean DAS–81419–2 × DAS–44406–6 (not treated with the intended herbicides), the test of difference identified statistically significant differences with the conventional counterpart for early stand count, days to maturity, plant height, 100-seed weight, yield and lodging. Of those endpoints, 100-seed weight fell under equivalence category IV while the other endpoints fell under equivalence category I or II.[13]
- For soybean DAS–81419–2 × DAS–44406–6 (treated with the intended herbicides), the test of difference identified statistically significant differences with the conventional counterpart for days to 50% flowering, days to maturity, plant height, 100-seed weight and lodging. Of those endpoints, 100-seed weight fell under equivalence category IV while the other endpoints fell under equivalence category I.[14]

As reported in Section 3.5.3.2, a different germination capacity was observed between soybean DAS–81419–2 × DAS–44406–6 and the conventional counterpart. This difference might be the cause of the reduction observed in early stand count for soybean DAS–81419–2 × DAS–44406–6 (not treated

[12] Early stand count, seedling vigour, days to 50% flowering, disease incidence, insect damage, days to maturity, lodging, plant height, final stand count, pod shattering, yield, 100-seed weight and number of seeds per plant (by calculation).

[13] The estimated mean values for 100-seed weight were: 14.8 g for the conventional counterpart; 13 g for the GM soybean (treated with conventional herbicides); and 12.5 g for the GM soybean (treated with the intended herbicides). The equivalence limits were (14, 18.4) g.

[14] [*Cross-refer to previous footnote for 100-seed weight*].

with the intended herbicides), which, however, fell under equivalence category I. The GMO Panel considered that the comparative analysis was not affected by the difference and lack of equivalence observed for 100-seed weight, as a similar outcome was not observed for the other yield components (yield and number of seeds per plant). Whether the difference in 100-seed weight can lead to an environmental adverse effect is considered in Section 3.7.1.

3.5.6. Compositional analysis

Soybean DAS–81419–2 × DAS-44406–6 seeds and forage harvested from nine sites (Table 4) were analysed for 107 constituents (11 in forage and 96 in seeds), including those recommended by OECD (OECD, 2012). Twenty seed constituents were excluded from the statistical analysis since more than 50% of the observations were below the limit of quantification.[15]

The statistical analysis was applied to a total of 87 constituents (76 in seed[16] and 11 in forage[17]). A summary of the outcome of the test of difference and the test of equivalence is presented in Table 5:

- For soybean DAS–81419–2 × DAS–44406–6 not treated with the intended herbicides, statistically significant differences with the conventional counterpart were identified for 32 endpoints (all in seeds). For two of them (acid detergent fibre (ADF) and phosphatidylinositol), the test of equivalence was not applied because the variability among the reference varieties was estimated to be zero, while lectin activity fell under equivalence category IV (Table 6). The other 29 endpoints fell under equivalence category I or II.
- For soybean DAS–81419–2 × DAS–44406–6 treated with the intended herbicides, statistically significant differences with the conventional counterpart were identified for 39 endpoints (34 in seeds and 5 in forage). The test of equivalence was not applied to four of the forage endpoints, while lectin activity and glutamic acid levels in seed fell under equivalence category III and IV, respectively (Table 6). The other 33 endpoints fell under equivalence category I or II.

The GMO Panel assessed all the significant differences between soybean DAS–81419–2 × DAS–44406–6 and its conventional counterpart, considering the potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties. Quantitative results for the endpoints showing significant differences between soybean DAS–81419–2 × DAS–44406–6 and its conventional counterpart and not falling under equivalence category I/II are given in Table 6.

[15] Phosphatidic acid, phosphatidylglycerol, phosphatidylserine, caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), myristoleic acid (C14:1), pentadecanoic acid (C15:0), pentadecenoic acid (C15:1), heptadecenoic acid (C17:1), γ-linolenic acid (C18:3), eicosadienoic acid (C20:2),eicosatrienoic acid (C20:3), arachidonic acid (C20:4), sodium, α-carotene, β-carotene, vitamin A from carotene and β-tocopherol.

[16] Ash, moisture, carbohydrates, crude fat, crude protein, acid detergent fibre, neutral detergent fibre, crude fibre, total dietary fibre, calcium, copper, iron, magnesium, manganese, phosphorus, potassium, selenium, zinc, alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C20:1), behenic acid (C22:0), α-tocopherol, ascorbic acid, δ-tocopherol, folic acid, γ-tocopherol, niacin, pantothenic acid, pyridoxine, riboflavin, thiamine, vitamin K, Gly m 1, Gly m 3, Gly m 4, Gly m 5, Gly m 6, Gly m 8, Gly m Bd 28 k, Gly m Bd 30 k, lectin activity, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phytic acid, raffinose, stachyose, total daidzein equivalent, total genistein equivalent, total glycitein equivalent and trypsin inhibitor.

[17] Ash, moisture, crude fat, crude protein, carbohydrates, crude fibre, nitrogen free extract, acid detergent fibre, neutral detergent fibre, calcium and phosphorus.

Table 5: Outcome of the comparative compositional analysis in forage and seeds of soybean DAS–81419–2 × DAS–44406–6. The table shows the number of endpoints in each category

		Test of difference[a]			
		Not treated[c]		Treated[c]	
		Not different	Significantly different	Not different	Significantly different
Test of equivalence[b]	Category I/II	40	29[d]	35	33[d]
	Category III/IV	5[e]	1[f]	5[e]	2[f]
	Not categorised	10[g]	2[h]	8[g]	4[h]
	Total endpoints	87		87	

(a): Comparison between soybean DAS-81419-2 × DAS-44406-6 and its conventional counterpart.
(b): Four different outcomes: 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). Not categorised means that the test of equivalence was not applied because of the lack of variation among the non-GM reference varieties.
(c): Not treated/treated with the intended herbicides.
(d): Endpoints with significant differences between soybean DAS-81419-2 × DAS-44406-6 and the conventional counterpart and falling in equivalence category I-II. In forage, untreated only: none. Treated only: calcium, crude fibre and neutral detergent fibre (NDF). Both treated and untreated: none. In seed, untreated only: acid detergent fibre (ADF), phosphatidylinositol and vitamin K. Treated only: copper, crude fat, crude protein, Gly m 4, Gly m 8, Gly m Bd 30 k, lysine and magnesium. Both treated and untreated: arachidic acid (C20:0), arginine, crude fibre, cystine, eicosenoic acid (C20:1), linoleic acid (C18:2), linolenic acid (C18:3), oleic acid (C18:1), palmitoleic acid (C16:1), potassium, stearic acid (C18:0), trypsin inhibitor, niacin, pantothenic acid and ascorbic acid.
(e): Endpoints falling in equivalence category III-IV and with no significant differences between soybean DAS-81419-2 × DAS-44406-6 and the conventional counterpart. In forage, none. In seed, untreated only: glutamic acid. Treated only: leucine. Both treated and untreated: Gly m 5, glycine, histidine and threonine.
(f): Endpoints with significant differences between soybean DAS-81419-2 × DAS-44406-6 and the conventional counterpart and falling in equivalence category III-IV. In forage, none. In seed, untreated only: none. Treated only: glutamic acid. Both treated and untreated: lectin activity. Quantitative results for these endpoints are reported in Table 6.
(g): Endpoints not categorised for equivalence and with no significant differences between soybean DAS-81419-2 × DAS-44406-6 and the conventional counterpart. In forage, untreated only: ADF, NDF, crude fibre and phosphorus. Treated only: none. Both treated and untreated: nitrogen free extract, carbohydrates and crude protein. In seed, untreated only: none. Treated only: ADF and phosphatidylinositol. Both treated and untreated: total dietary fibre, proline and selenium.
(h): Endpoints not categorised for equivalence and with significant differences between soybean DAS-81419-2 × DAS-44406-6 and the conventional counterpart. In forage, untreated only: none. Treated only: ADF, NDF, crude fibre and phosphorus. Both treated and untreated: none. In seed, untreated only: ADF and phosphatidylinositol. Treated only: none. Both treated and untreated: none. Quantitative results for these endpoints are reported in Table 6.

Table 6: Quantitative results (estimated means and equivalence limits) for endpoints with significant differences between soybean DAS–81419–2 × DAS–44406–6 and the conventional counterpart and not falling under equivalence category I/II (see Table 5)

	Endpoint	Soybean DAS–81419–2 × DAS–44406–6		Conventional counterpart	Non-GM reference varieties	
		Not treated[a]	Treated[a]		Mean	Equivalence limits
Forage	ADF (% dw)	25.96	25.24*	26.51	25.63	–
	NDF (% dw)	32.88	31.43*	32.91	32.35	–
	Crude fibre (% dw)	22.89	22.20*	23.56	23.36	–
	Phosphorus (mg/100 g dw)	289.7	285.3*	296.3	309.1	–
Seed	ADF (% dw)	12.73*	12.59	12.2	12.53	–
	Glutamic acid (% AA)	17.21	17.12*	17.26	17.72	17.36–18.10
	Phosphatidylinositol (% dw)	0.06583*	0.06547	0.06366	0.06594	–
	Lectin activity (HU/mg protein)[b]	10.23*	9.46*	8.03	5.34	3.87–7.36

For the GM soybean, significantly different values are marked with an asterisk, while the outcomes of the test of equivalence are differentiated by greyscale backgrounds. Light and dark grey backgrounds correspond to equivalence category III and IV, respectively. A white background is used when the test of equivalence is not applied.
ADF: Acid detergent fibre; dw: dry weight; NDF: neutral detergent fibre; –: test of equivalence not applied because of the lack of variation among the non-GM reference varieties.
(a): Not treated/treated with the intended herbicides.
(b): A UPLC-MS/MS method was also used to measure soybean agglutinin in treated and not treated DAS-81419-2 × DAS-44406-6 soybean, a conventional counterpart, and 10 non-GM commercial soybean lines (additional information September 2018). Mean concentration of the conventional counterpart was 5.56 μg/mg dw, while concentrations of 6.83 μg/mg dw and 7.02 μg/mg dw were reported for the not treated and treated DAS-81419-2 × DAS-44406-6 soybean, respectively. The outcome of the equivalence test was consistent with the outcome of the measurements using the haemagglutinin test.

3.5.7. Conclusions of the comparative analysis

Taking into account the natural variability observed for the set of non-GM reference varieties, the GMO Panel concludes the following:

None of the differences identified in the agronomic and phenotypic characteristics between soybean DAS814192 × DAS444066 and its conventional counterpart needs further assessment, except for those in regard to 100-seed weight. These differences are further assessed for their potential environmental impact in Section 3.7.1.

None of the differences identified in forage and seed composition between the two-event stack soybean and its conventional counterpart needs further assessment regarding food and feed safety, except for the levels of ADF, NDF, crude fibre and phosphorus in forage and ADF, phosphatidylinositol, glutamic acid and lectin activity in seeds. The safety assessment is done in Section 3.6.

3.6. Food and feed safety assessment[18]

3.6.1. Effects of processing

Soybean DAS-81419-2 × DAS-44406-6 will undergo existing production processes used for conventional soybean. Based on this, the characteristics of the intended traits and the outcome of the comparative assessment, the processing of the double-event stack soybean into food and feed products is not expected to result in products being different from those of conventional non-GM soybean varieties. The compositional analysis identified an increase of around 18–27% in lectin activity in soybean DAS–81419–2 × DAS–44406–6 as compared to its conventional counterpart (not treated and treated).[19] As described in the scientific opinion of the single event DAS-44406-6 (EFSA GMO Panel, 2017b), food and feed processing generally reduces the content and/or activity of most soybean endogenous anti-nutrients, including lectins (Liener, 1994; Duranti and Gius, 1997; OECD, 2012; Shi et al., 2018). Soybean DAS-81419-2 × DAS-44406-6 toasted meal fed to rats and broilers in respective feeding studies, was analysed for lectin activity, showing values below the limit of quantification.[20]

3.6.2. Influence of temperature and pH on newly expressed proteins

The effects of temperature and pH on the newly expressed proteins in this two-event stack soybean have been previously evaluated by the GMO Panel (Table 1).

3.6.3. Toxicology

3.6.3.1. Testing of newly expressed proteins

Five proteins (AAD-12, Cry1Ac, Cry1F, PAT and 2mEPSPS) are newly expressed in soybean DAS–81419–2 × DAS–44406–6 (Section 3.4.1). The GMO Panel has previously assessed these proteins in the context of the single events (Table 1), and no safety concerns were identified for humans and animals. The unpublished toxicological studies provided in the context of this application (see Appendix A) did not change this conclusion. The GMO Panel is not aware of any other new information that would change this conclusion. The potential for a functional interaction between the proteins newly expressed in soybean DAS-81419-2 × DAS-44406-6 has been assessed with regard to human and animal health. The two

[18] Dossier: Part II - Sections 1.3.6, 1.4, 1.5, 1.6 and 2; additional information: 15/5/17, 10/1/18, 14/5/18, 27/8/18, 21/9/18, 4/12/18, 18/12/18, 12/2/19, 15/2/19, 4/11/19, 15/4/20, 15/6/20 and 10/8/20.
[19] Measurements using a haemagglutinin test.
[20] Dow AgroSciences LLC, Study ID: 130454, Meal characterization of DAS-81419-2 × DAS-44406-6 soybean.

insecticidal proteins (Cry1F and Cry1Ac) are delta endotoxins acting through cellular receptors found in target insect species. It is reported that the gastrointestinal tract of mammals, including humans, lacks receptors with high specific affinity to Cry proteins (Hammond et al., 2013; Koch et al., 2015). The AAD-12, PAT and 2mEPSPS proteins are enzymes that catalyse distinct biochemical reactions and act on unrelated substrates with high substrate specificity. On the basis of the known biological function of the individual newly expressed proteins (Table 3), there is currently no expectation for possible interactions relevant to the food and feed safety of soybean AAD-12, PAT and 2mEPSPS. *In vitro* protein degradation studies on AAD-12, Cry1Ac, Cry1F, PAT and 2mEPSPS proteins have been previously evaluated by the EFSA GMO Panel and no indications of safety concerns were identified (Table 1). The GMO Panel concludes that there are no safety concerns to human and animal health related to the newly expressed proteins AAD-12, Cry1Ac, Cry1F, PAT and 2mEPSPS in soybean DAS–81419–2 × DAS–44406–6.

3.6.3.2. Testing of new constituents other than proteins

No new constituents other than newly expressed proteins have been identified in soybean DAS–81419–2 × DAS–44406–6. Therefore, no further food and feed safety assessment of components other than the newly expressed proteins is required.

3.6.3.3. Information on altered levels of food and feed constituents

The levels of ADF, NDF, crude fibre and phosphorus in forage and ADF, phosphatidylinositol, glutamic acid and lectin activity in seeds were significantly different in soybean DAS-81419-2 × DAS-44406-6 when compared with its conventional counterpart and showed a lack of equivalence (or could not be categorised) with the non-GM reference varieties (Section 3.5.6).

Taking into account the biological characteristics and functions of these compounds, the observed differences are considered of no toxicological concern. In particular, with regard to the increased lectin activity (~ 25%) observed in soybean DAS-81419-2 × DAS-44406-6 as compared to the conventional counterpart, the GMO Panel assessed these data taking into account previous assessments of the GMO Panel on the single events (EFSA GMO Panel, 2016, 2017b) and the available information on this compound relevant for safety. Considering that i) the increase in the lectin activity was comparable between that observed in this stack (~ 25%) and in the single event (~ 31%); and ii) that there is no new information on the safety of lectins that would challenge previous opinions on the single events of the GMO Panel, the increase in lectin activity observed in soybean DAS-81419-2 × DAS-44406-6 is considered of no safety concern. Further information on safety of these compounds is provided in Sections 3.6.4 and 3.6.6.

3.6.3.4. Testing of the whole genetically modified food and feed

Based on the outcome of the molecular characterisation, comparative analysis and toxicological assessment, no indication of findings relevant to food and feed safety related to the stability and expression of the inserts or to interactions between the transformation events, and no modifications of toxicological concern in the composition of soybean DAS-81419-2 × DAS-44406-6 have been identified (see Sections 3.4.3 and 3.5.7). Therefore, animal studies on food and feed derived from the two-stack soybean are not necessary (EFSA GMO Panel, 2011a).

The applicant provided a total of four 90-day studies on the whole food and feed; in this section, the GMO Panel reports in detail the assessment of the legally requested and compliant study (Table 7).

Table 7: Summary of 90-day studies provided in application EFSA-GMO-NL-2016-132

Event	Submission to EFSA	GMO Panel Assessment
Soybean DAS-44406-6	Spontaneous, Main dossier	Assessed by the GMO Panel (2017b) and found not to raise safety concerns; since the test material was not treated with all the intended herbicides, this study is considered not in line with Reg. (EU) No 503/2013
Soybean DAS-81419-2	Spontaneous, Main dossier	See Appendix A
Soybean DAS-81419-2 × DAS-44406-6	Spontaneous, Main dossier	See Appendix A
Soybean DAS-81419-2 and Soybean DAS-44406-6	Additional information provided upon EFSA 's clarification request on the legally requested studies on the single-event soybeans	Reported in detail in Section 3.6.3.4 of this Scientific Opinion

90-day study on soybean DAS-81419-2 and soybean DAS-44406-6

In this 90-day study, the applicant tested groups of rats given diets containing DAS-81419-2 or DAS–44406-6 soybean and comparing these with one control group. This study is adapted from OECD Test Guideline 408 (OECD, 1998) and complies with the principles of Good Laboratory Practice, except for the lack of analytical determination of concentration, homogeneity and stability of the test item in the formulated diets. It is recognised that it may not always be technically possible to generate information on homogeneity and concentration for a test item administrated or formulated, and the lack of such data and its impact on the validity of a study should be justified (OECD, 2018). The GMO Panel acknowledges that there are no practical methods available to analytically determine these for complex test items such as soybean meal in formulated diets and considers adequate the application of proper diet preparation procedures and regular evaluations of the mixing methods. Based on the information received from the applicant, the GMO Panel considers that the diet preparation procedures in place in the facilities where the diets for this study were prepared guaranteed their homogeneity and the proper concentration of the respective test or control items. As regards the stability of the test item (soybean meal, oil and hulls) in the diets, the applicant considers that, in accordance with product expiration declared by the diet manufacturer, the constituents of the diets used in these studies are stable for the duration of the treatment. The GMO Panel considers this justification acceptable. In addition, the GMO Panel notes that the diets were prepared and analysed in an ISO certified facility.

A total of 256 Crl:CD(SD) rats (128 per sex) were randomly allocated to eight treatment groups (one control group, one low and one high dose DAS-81419-2, one low and one high dose DAS-44406-6 and three reference groups, n = 16/sex per group) using a stratified complete block design. The test groups were given diets containing approximately 15% (w/w) defatted toasted meal, 1% hulls and 1.35% oil (low dose) and 30%, 2% hulls and 2.7% oil (high dose) from DAS-81419-2 or DAS-44406-6, respectively. The DAS-44406-6 source material was sprayed with the intended herbicides.[21] The DAS–81419-2 source material was not treated with glufosinate (the *pat* gene is used only as selectable marker) and it is considered adequate for this study by the GMO Panel, since consistent with the assessment of the single event (EFSA GMO Panel, 2016). The control material (Maverick) is appropriate. The seeds used to produce the test and control materials were sent to the processing facility in about 1 month from harvest, then maintained at room temperature for about 1 month and finally processed into defatted toasted meal, oils and hull. The identity of DAS-81419-2 and DAS-44406-6 test materials (meals) was confirmed by PCR. Balanced diets were prepared according to the specifications for PMI Certified Rodent LabDiet#5002, but were not analysed for the presence of the respective events; chain of custody data confirmed the identity of the diets. Test items, control and reference materials, as well as test, control and reference diets were analysed for proximates, amino acids, minerals, mycotoxins, pesticides and antinutrients). In-life procedures and observations and terminal procedures were conducted in accordance with OECD Test Guideline 408 (OECD, 1998).

The statistical analysis was conducted separately for DAS-81419-2 and DAS-44406-6. For each of the two events, rats consuming the low- and high-dose test diets were compared with those consuming the control diet. For continuous parameters, a linear mixed model was applied to data for individual animals for the two sexes combined (fixed effects: diet, sex and sex-by-diet interaction; random effects: block-within-sex and cage). Test-control comparisons were done both across sexes and separately for males and females; in case a significant sex-by-diet interaction was identified, only the sex-specific results were considered for the assessment. The model was modified as needed for the analysis of sex-specific endpoints and cage-level data (food consumption and food efficiency). The data for the three reference groups were included in the analysis and used to calculate ranges of variability for the parameters.

There were no diet-related incidents of mortality or clinical signs. No test diet-related adverse findings were identified in any of the investigated parameters. A small number of statistically significant findings were noted, but these were not considered adverse effects of treatment for one or more of the following reasons:

- were present at the low dose but not in the high-dose groups;
- were within the normal variation for the parameter in rats of this age;
- were of small magnitude;

[21] DAS-4406-6: 2,4-D (2,4-dichlorophenoxyacetic acid), and glufosinate (L-phosphinothricin) ammonium and glyphosate (N-(phosphonomethyl) glycine).

- were identified at only a small number of time intervals with no impact on the overall value;
- exhibited no consistent pattern with related parameters or end-points.

Detailed description of statistically significant findings identified in rats given diets containing DAS–81419–2 and DAS-44406-6 test materials is reported in Appendix D.

No gross pathological findings related to the administration of the test diets were observed at necropsy in rats given diets containing DAS-81419-2 soybean, and the microscopic examinations of selected organs and tissues did not identify relevant differences in the incidence and severity of the histopathological findings related to the administration of the test diet.

With regard to the groups given diets containing DAS-44406-6 soybean test materials, at post-mortem examination, gross and/or histopathological findings were reported in the testes and epididymides of four rats from the high-dose group. One high-dose rat with very low testes weights was, as expected, identified at gross examination as having testes of decreased size. One other high-dose rat was found to have a flaccid testis. Degeneration of seminiferous tubules was reported in the testes of three high-dose rats and one control rat (not significant $p = 0.6$ Fisher's exact test) at histopathological examination. Decreased and/or degenerative spermatic elements were found in the epididymides of four high-dose rats compared with none in controls (not significant $p = 0.1$ Fisher's exact test). There were no effects reported on the pathology of the seminal vesicles and prostate inflammation was more prevalent in controls (8/16) than in the high-dose group (6/16). The findings in the testes and epididymides did not show a statistically significant difference between groups, they occur spontaneously in rats of this strain and age and there were no increases in lesions in other parts of the male reproductive system, and therefore are considered not to be an adverse effect of treatment. No other gross findings related to the administration of the test diet were observed at necropsy. The microscopic examinations of selected organs and tissues (high dose and control diets) did not identify relevant differences in the incidence and severity of the histopathological findings related to the administration of the test diet.

The GMO Panel concludes that this study is in line with the requirements of Regulation (EU) No 503/2013 and that no treatment-related adverse effects were observed in rats after feeding diets including soybean DAS-81419-2 or soybean DAS-44406-6 (up to 30% defatted toasted meal, 2% hulls and 2.7% oil) for 90 days.

3.6.4. Allergenicity

For the allergenicity assessment, a weight-of-evidence approach was followed, taking into account all the information obtained on the newly expressed proteins, as no single piece of information or experimental method yields sufficient evidence to predict allergenicity and adjuvanticity (Codex Alimentarius, 2009; EFSA GMO Panel, 2011a; Commission Regulation (EU) No 503/2013).

3.6.4.1. Assessment of allergenicity of the newly expressed proteins

For allergenicity, the GMO Panel has previously evaluated the safety of AAD-12, Cry1Ac, Cry1F, PAT and 2mEPSPS proteins individually, and no evidence of allergenicity was identified in the context of the applications assessed (Table 1). EFSA published a technical report on the safety assessment of Cry1Ac in GM crops confirming previous EFSA opinions (EFSA, 2018). No new information on allergenicity of the proteins newly expressed in this two-event stack soybean that might change the previous conclusions of the GMO Panel has become available. Based on the current knowledge, and as there is no evidence of allergenicity of the newly expressed proteins, there are no expected concerns of allergenicity as a consequence of their interaction in this two-event stack soybean.

The GMO Panel has previously evaluated the safety of the newly expressed proteins, and no evidence of adjuvanticity was identified in the context of the applications assessed (Table 1). More recently, this aspect has been discussed in detailed by EFSA (EFSA, 2018; Parenti et al., 2019). To date, there is no evidence for adjuvanticity in the GMOs assessed by the Panel. This two-event stack soybean has comparable levels of the individual *Bt* proteins than those in the respective single soybean events (see Section 3.4.3). The GMO Panel did not find indications that the *Bt* proteins at the levels expressed in this two-event stack soybean might be adjuvants able to enhance an allergic reaction.

3.6.4.2. Assessment of allergenicity of GM plant products

Soybean is considered a common allergenic food[22] (OECD, 2012). Therefore, any potential change in the endogenous allergenicity of the GM plant should be assessed (Regulation (EU) No 503/2013). For such assessment, the applicant included in the comparative analysis specific allergens relevant for soybean (Section 3.5.6) quantified using liquid chromatography with tandem mass spectrometry, which has been previously considered acceptable (EFSA GMO Panel, 2010c, 2017b; Fernandez et al., 2013; Selb et al., 2017). The applicant also referred to the Kunitz trypsin inhibitor as a potential soybean allergen, which is an anti-nutrient and as such it is already assessed in the compositional analysis (Section 3.5.6). These allergens were selected based on the list of potential soybean allergens described in the pertinent OECD document (OECD, 2012) and a scientific rational supporting their selection was provided by the applicant and considered acceptable by the GMO Panel. No changes in the levels of endogenous allergens raising concern are identified by the GMO Panel.

The GMO Panel also noted an increase in lectin content in soybean DAS-81419-2 × DAS-44406-6 when compared with that in its conventional counterpart (see Section 3.5.6). Soybean lectins were previously suggested as potential allergens (L'Hocine and Boye, 2007). However, the clinical evidence of soybean lectins as relevant allergens is weak/non-existent (Ladics et al., 2014; Selb et al., 2017; EFSA GMO Panel, 2017b). Briefly, there are few publications that showed IgE-binding activity against soybean lectins with sera from allergic individuals, but with no clinical relevance described (Rougé et al., 2010; Batista et al., 2007; Lu et al., 2018). Other publications report an absence of evidence of IgE-binding activity to tested soybean lectins (Ogawa et al., 1991; Shibasaki et al., 1992). The GMO Panel also considered the observed increase in the context of previous assessments of the single events (see Section 3.6.3; EFSA GMO Panel, 2016, 2017b). Considering all this information, the increase of lectin content in soybean DAS-81419-2 × DAS-44406-6 as compared with its conventional counterpart does not raise concerns to the GMO Panel.

In the context of this application, the GMO Panel considers that there is no evidence that the genetic modification might substantially change the overall allergenicity of the two-event stack soybean when compared with that of the conventional counterpart and the non-GM reference varieties tested.

3.6.5. Dietary exposure assessment to new constituents

In line with Regulation (EU) No 503/2013, the applicant provided dietary exposure estimates to AAD–12, Cry1Ac, Cry1F, PAT and 2mEPSPS proteins newly expressed in soybean DAS-81419-2 × DAS-44406–6. Dietary exposure was estimated based on protein expression levels reported in this application for the double-event stack soybean treated with the intended herbicides, the current available consumption data and feed practices, the foods and feeds currently available in the market and the described processing conditions.

Table 8 describes the protein expression levels derived from replicated field trials in USA during 2012 (nine locations) used to estimate both human and animal dietary exposure to AAD-12, Cry1Ac, Cry1F, PAT and 2mEPSPS proteins.

Table 8: Mean values (n = 36, μg/g dry weight) for newly expressed proteins in seeds and forage from DAS-81419-2 × DAS-44406-6 soybean treated with the intended herbicides[(a)]

Protein	Tissue/developmental stage	
	Seeds/R8 (μg/g dry weight)	Forage/R3 (μg/g dry weight)
2mEPSPS	12.09	507.43
AAD-12	29.62	44.53
PAT	2.59	7.36
Cry1F	14.85	12.62
Cry1Ac	1.41	3.99

(a): Intended herbicides: 2,4-D, glufosinate and glyphosate.

[22] 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.

3.6.5.1. Human dietary exposure

As per request of the GMO Panel, chronic and acute dietary exposure to the newly expressed proteins in DAS-81419-2 × DAS-44406-6 soybean was provided. The applicant followed the methodology described by EFSA to estimate dietary exposure in high consumers using summary statistics (EFSA, 2015).

Dietary exposure was estimated across different European countries on different population groups: young population (toddlers, 'other children'), adolescents, adult population (adults, elderly and very elderly). Consumption figures for the relevant commodities (soya bread, protein supplements, textured soy protein, soya drink, tofu etc.) were retrieved from the EFSA Comprehensive European Food Consumption Database.[23] Soybean oil was excluded from the assessment since no proteins are expected to be present in the oil.

Mean protein expression values on fresh weight basis are considered as the most adequate to estimate human dietary exposure (EFSA, 2019a). However, dietary exposure was provided using expression values on dry weight basis; this results in more conservative exposure estimations which is considered acceptable. The GMO Panel also noticed that for 2mEPSPS protein, an exceptionally high variation in the expression values was reported (mean ± SD = 12.09 ± 13.65; range 4.15–62.0 μg/g), particularly due to the high expression levels determined in one of the field sites. The applicant used the mean expression values for 2mEPSPS protein when estimating acute dietary exposure, although the most adequate approach would be to use the average of the site with the highest concentrations (EFSA, 2019a). The GMO Panel accepted this approach considering that no safety concerns are identified for this protein (Section 3.6.3.1) and that an overly conservative scenario with 100% replacement is used overall. Different factors were considered to estimate the amount of soybean in the consumed commodities before assigning AAD-12, Cry1Ac, Cry1F, PAT and 2mEPSPS protein levels to the relevant commodities. No losses in the newly expressed proteins during processing were considered, except for soybean oil which was eventually excluded from the exposure estimations.

The highest acute dietary exposure was in the age class 'Adults' with Cry1Ac, Cry1F, PAT, AAD-12, and 2mEPSPS with estimates of 0.019 mg/kg body weight (bw) day, 0.21 mg/kg bw day, 0.036 mg/kg bw day, 0.41 mg/kg bw day, and 0.17 mg/kg bw per day, respectively.

The highest chronic dietary exposure was in the age class 'Adolescents' with Cry1Ac, Cry1F, PAT, AAD–12, and 2mEPSPS with estimates of 0.004 mg/kg bw day, 0.04 mg/kg bw day, 0.007 mg/kg bw day, 0.08 mg/kg bw day and 0.03 mg/kg body weight/day, respectively.

Furthermore, the current consumption data on 'Pollen supplements' available in the EFSA Consumption Database[24] indicate that additional dietary exposure to the newly expressed proteins might occur under the assumption that these supplements contain pollen from soybean DAS-81419-2 × DAS-44406-6. Since no data on the presence of newly expressed proteins in pollen were available, dietary exposure from this source was not estimated.

3.6.5.2. Animal dietary exposure

Dietary exposure to AAD-12, Cry1Ac, Cry1F, PAT and 2mEPSPS proteins in soybean DAS-81419-2 × DAS-44406-6 was estimated across different animal species as described below, assuming the consumption of soybean products commonly entering the feed chain (i.e. soybean meal and protein concentrates, hulls and forage).

A conservative scenario with 100% replacement of conventional soybean products by the soybean DAS–81419-2 × DAS-44406-6 products was considered.

Mean levels (dry weight) of AAD-12, Cry1Ac, Cry1F, PAT and 2mEPSPS proteins in seeds and forage from soybean DAS-81419-2 × DAS-44406-6 used for animal dietary exposure are those listed in Table 8.

The applicant estimated dietary exposure to AAD-12, Cry1Ac, Cry1F, PAT and 2mEPSPS proteins via the consumption of soybean meal in dairy cow, cattle for fattening, pig for fattening, sow lactating, sheep/goat, chicken for fattening, laying hen, turkey for fattening, salmon, dog, and cat, and forage in laying hen, based on body weights and daily feed intakes as recommended by EFSA (EFSA FEEDAP

[23] Summary statistics from the EFSA Comprehensive European Food Consumption Database accessed in May 2018. Available online: https://www.efsa.europa.eu/en/food-consumption/comprehensive-database

[24] EFSA Comprehensive European Food Consumption Database (accessed in March 2020). Available online: https://www.efsa.europa.eu/en/food-consumption/comprehensive-database

Panel, 2017) and inclusion rates of soybean meal and forage in diets, as recommended by OECD for the EU livestock population (OECD, 2013). Inclusion rates in salmon diets (12%) were based on FAO recommendations,[25] while those in cats and dog (30% and 15%) on Purina recommendation[26] (personal communication). To estimate the mean levels of AAD-12, Cry1Ac, Cry1F, PAT and 2mEPSPS proteins in soybean meal, the mean concentrations of these proteins in seeds were used, assuming that no losses of newly expressed proteins occur during processing. Estimated dietary exposures based on the consumption of soybean meal are reported in Table 9.

Table 9: Dietary exposure to Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins in food-producing and non-food producing animals, based on the consumption of soybean meal

	Dietary exposure (µg/kg body weight per day)[(a)]				
	Cry1F	**Cry1Ac**	**AAD-12**	**PAT**	**2mEPSPS**
Cattle for Fattening	59	5.6	120	10	48
Dairy Cow	120	11	230	20	94
Sheep/Goat	74	7.1	150	13	60
Sow Lactating	130	13	270	23	110
Pig for Fattening	160	16	330	29	130
Chicken for Fattening	470	45	940	82	380
Laying Hen	200	19	390	34	160
Turkey for Fattening	390	37	790	69	320
Salmon	32	3	64	5.6	26
Dog	38	3.6	76	6.6	31
Cat	89	8.5	180	16	73

(a): The GMO Panel considers that after extraction of the oil, crude protein in soybean meal increases by a factor of 1.28, based on the protein content of soybean meal relative to soybean seed (OECD, 2009), assuming that no protein is lost during the processing. Therefore, the above-reported values for the newly expressed proteins present in meal should be adjusted accordingly.

To further integrate the assessment, the GMO Panel estimated the animal dietary exposure to AAD-12, Cry1Ac, Cry1F, PAT and 2mEPSPS proteins via the consumption of forage in dairy cattle, and of other soybean products (i.e. protein concentrates and hulls) entering the feed chain, considering the concerned animals for each feed commodity.

Laying hen and dairy cattle for forage

Consumption of soybean forage is based on estimates for animal body weight and daily feed intake (OECD, 2009), and inclusion rates of soybean forage in animal diets (OECD, 2012). Estimated dietary exposures based on the consumption of soybean forage are reported in Table 10.

Table 10: Dietary exposure to Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins in food-producing animals, based on the consumption of forage

	Dietary exposure (µg/kg bw per day)				
	Cry1F	**Cry1Ac**	**AAD-12**	**PAT**	**2mEPSPS**
Laying Hen[(a)]	67	21	240	39	2,690
Dairy Cow[(b)]	97	30	342	56	3,900

(a): Estimations as provided by the applicant.
(b): Estimations as provided by EFSA.

Beef and dairy cattle, rams, lambs, breeding and finishing pigs, broiler and layer chickens for hulls

Consumption of soybean hulls is based on estimates for animal body weight, daily feed intake and inclusion rates of hull in animal diets (OECD, 2009, 2013). To estimate the mean newly expressed protein levels in soybean hulls, a factor of 0.3-fold was applied based on the protein content of

[25] FAO (FAO Food and Agricultural Organisation of the United Nations), 2017. Atlantic salmon - Nutritional requirements. Available online: http://www.fao.org/fishery/affris/species-profiles/atlantic-salmon/nutritional-requirements/en/
[26] Additional information: 10/8/2020.

soybean hulls relative to soybean seed (OECD, 2012), assuming that no losses of these proteins occur during processing. Estimated dietary exposures based on the consumption of soybean hulls are reported in Table 11.

Table 11: Dietary exposure to Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins in food-producing animals, based on the consumption of soybean hull

	Dietary exposure (μg/kg bw per day)				
	Cry1F	**Cry1Ac**	**AAD-12**	**PAT**	**2mEPSPS**
Beef cattle	10.668	1.015	21.326	1.864	8.683
Dairy cattle	17.096	1.626	34.176	2.988	13.915
Ram	29.633	2.820	59.240	5.180	24.120
Lamb	37.782	3.595	75.531	6.604	30.753
Breeding pigs	10.257	0.976	205.061	1.793	8.349
Finishing pigs	13.335	1.269	26.658	2.331	10.854
Broiler	15.688	1.492	31.362	2.742	12.769
Layer	15.206	1.447	30.399	2.658	12.377

Piglets for protein concentrates

Consumption of soybean protein concentrates in piglet is based on estimates for animal body weight, daily feed intake (EFSA FEEDAP Panel, 2017; EFSA, 2019b) and inclusion rates of protein concentrates in diets (7%) (Guzmán et al., 2016). To estimate the mean newly expressed protein levels in soybean protein concentrates, a factor of 1.75-fold was applied based on the protein content of soybean protein concentrates (70%), relative to soybean seed (OECD, 2012), assuming that no losses of these proteins occur during processing. Estimated dietary exposures to Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins based on the consumption of protein concentrates is, respectively, 800.184, 7.576, 159.636, 13.952 and 64.988 μg/kg bw per day.

3.6.6. Nutritional assessment of endogenous constituents

The intended traits of soybean DAS–81419–2 × DAS–44406–6 are herbicide tolerance and insect resistance, with no intention to alter nutritional parameters. However, several compounds (ADF, NDF, crude fibre and phosphorus in forage, and lectin activity, ADF, phosphatidylinositol and glutamic acid in seeds) were significantly different from its conventional counterpart and showed a lack of equivalence with the set of non-GM reference varieties/could not be categorised (Section 3.5.6). The biological relevance of these compounds, the role of soybean as contributor to their total intake and the magnitude and direction of the observed changes were considered during the nutritional assessment.

3.6.6.1. Human nutrition

The human nutritional assessment covered the observed changes in the levels of ADF, glutamic acid phosphatidylinositol and lectin activity in seeds (see Section 3.5.6).

In the context of human nutrition, fibre is referred to as dietary fibre, which primarily includes non-starch polysaccharides (mainly cellulose, hemicelluloses, pectins and other hydrocolloids) and lignin (EFSA NDA Panel, 2010). Therefore, the observed increase (~ 4%) in ADF (cellulose and lignin) implies an increased intake of dietary fibre. Dietary fibre is present in certain soybean-derived foods (e.g. soybean flour), while it is almost absent in other products such as tofu and soya milk. Although evidences link high consumption of fibre (above 25 g per day) with health benefits (EFSA NDA Panel, 2010), foods from soybean are, overall, not major contributors to total dietary fibre intake. Therefore, the increase in ADF is not considered of nutritional relevance.

The relatively small decrease in glutamic acid (< 1%) which is not an essential amino acid does not represent nutritional concerns.

Phosphatidylinositol is one of the main phospholipids of the soybean lecithin (Wendel, 1995), which is an authorised food additive recently re-evaluated (EFSA ANS Panel, 2017). Neither chronic nor acute toxicity has been identified for phosphatidylinositol using animal studies (Honda et al., 2009). Considering this information and its relatively low concentration, phosphatidylinositol levels in soybean DAS-81419-2 × DAS-44406-6 do not represent nutritional concern.

The activity levels of lectins in the double-stack soybean were around 18–27% higher (not treated and treated) as compared to its conventional counterpart.[22] The risk assessment of the single event DAS-44406-6 already concluded that an increase of up to 31% in the GM-crop as compared to its conventional counterpart does not represent any concern (EFSA GMO Panel, 2017b). Lectin dietary intake was not estimated during the risk assessment of the single event DAS–44406–6 based on the information considered during the toxicological assessment: the toxicity of raw soybean lectins is low compared to other commonly consumed legumes, industrial and traditional home processing practices are known to considerably reduce lectin content and/or activity in legumes (including soybean), the observed increase was considered in the context of the high variability reported for lectin activity and lectin protein content in raw soybean (Nasi et al., 2009; EFSA GMO Panel, 2017b). There is no new information on lectins that might challenge the approach followed in previous assessments; the conclusions of the risk assessment of the single event DAS-44406-6 remain valid for soybean DAS–81419–2 × DAS–44406–6.

3.6.6.2. Animal nutrition

Dietary fibre is considered essential for animal health due to its influence on gastrointestinal tract physiology. Firstly, by physical 'structuring' of digesta, which is relevant to feelings of satiety and control of food intake. Secondly, by modulation of digestive processes such as those which control transit time, which contribute to the control of circulating glucose and lipid levels, and lastly, by acting as an energy source for microbial fermentation, particularly in the large intestine. Ruminant's diet consists of feed materials of plant origin or their by-products which contains variable amount of fibre which ruminants may use as energy source through degradation by rumen microbes (e.g. anaerobic bacteria, protozoa and fungi). In contrast, monogastric animals and poultry cannot use the fibre as energy source, because they lack gastric bacterial fermentation and endogenous enzymes capable to digest fibre, although, up to certain amount, microbial digestion may happen in the large intestine. The magnitude of the differences in percentages (both increase or decrease) observed in crude fibre, NDF (hemicellulose, cellulose and lignin) and ADF (cellulose and lignin) in GM forage and ADF in GM seeds does not represent a safety issue for animals, and the nutritional impact in feeds is considered negligible. Crude fibre, NDF and ADF are fractions of the feed material characterised in the proximate analysis to proper formulate balanced diets and rations for animals taking into account to meet animal nutritional requirements.

Among minerals, phosphorus is considered a major element important in animal nutrition; the magnitude of the decreased level in GM forage does not represent per se a safety issue for animals, and the nutritional impact in feeds is considered negligible. Phosphorus occurs in plants mainly in the form of phytates and a minimal amount of its total concentration is utilisable by monogastric animals and poultry. In addition, animal diets and rations are balanced with macro minerals and trace elements, taking into account specific animal nutritional requirements.

Glutamic acid is not an essential amino acid, and can be synthetised in the organism; however, Wu (2014) argues that non-essential amino acids, including glutamine or glutamate, must be present in the diet of animals to optimise their survival, growth, development, reproduction and health. The magnitude of the decrease observed in seeds does not pose an issue for animal nutrition, also considering that animal's diet is balanced in order to provide the correct amount of essential and non-essential amino acids, by considering the different content of amino acids in feeds, and, eventually, by adding synthetic amino acids.

The increased levels of phosphatidylinositol in soybean seeds DAS-81419-2 × DAS-44406-6 compared to its conventional counterpart, is considered negligible and does not pose an issue for animal nutrition considering its widespread presence in eukaryotic animals and plants cells, and the limited magnitude of the difference observed.

The increased levels of lectins in soybean seeds DAS-81419-2 × DAS-44406-6 compared to its conventional counterpart (~ 25%), does not represent a concern for animal nutrition. The magnitude of this increase is comparable or even lower than previously assessed in seeds from the single event DAS-44406-6 and its conventional counterpart (~ 31%), for which nutritional concerns were not identified by the GMO Panel (EFSA GMO Panel, 2017b).

3.6.7. Conclusion of the food and feed safety assessment

The newly expressed proteins Cry1Ac, Cry1F, PAT, AAD-12 and 2mEPSPS in soybean DAS-81419-2 × DAS-44406-6 do not raise safety concerns for human and animal health. Interactions between the

newly expressed proteins Cry1Ac, Cry1F, PAT, AAD-12 and 2mEPSPS raising food and feed safety concerns (in terms of toxicology, allergenicity and adjuvanticity) are not expected. There is no evidence that the genetic modification might change the overall allergenicity of the two-event stack soybean. Based on the outcome of the animal and human nutritional assessments, the consumption of soybean DAS-81419-2 × DAS-44406-6 does not represent any nutritional concern, in the context of the scope of this application.

3.7. Environmental risk assessment[27]

Considering the scope of application EFSA–GMO–NL–2016–132, which excludes cultivation, the environmental risk assessment (ERA) of soybean DAS–81419–2 × DAS–44406–6 mainly takes into account: (1) the exposure of microorganisms to recombinant DNA in the gastrointestinal tract of animals fed GM material and of microorganisms present in environments exposed to faecal material of these animals (manure and faeces); and (2) the accidental release into the environment of viable soybean DAS–81419–2 × DAS–44406–6 seeds during transportation and/or processing (EFSA GMO Panel, 2010a).

3.7.1. Persistence and invasiveness of the GM plant

Cultivated soybean (*Glycine max* (L.) Merr.) is a species in the subgenus *Soja* of the genus *Glycine*. The species originated from eastern Asia and is a highly domesticated crop, generally unable to survive in the environment without proper management (Lu, 2005).

Occasional feral GM soybean plants may occur outside cultivation areas, but survival is limited mainly by a combination of low competitiveness, the absence of a dormancy phase and susceptibility to plant pathogens and cold climatic conditions (OECD, 2000). Soybean can grow as volunteers and 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, as for the same reasons mentioned above, soybean seeds usually do not survive during the winter (Owen, 2005). Thus, the establishment and survival of feral and volunteer soybean in the EU is currently limited and transient.

It is unlikely that the intended traits of soybean DAS–81419–2 × DAS–44406–6 will provide a selective advantage to soybean plants, except when they are exposed to 2,4–D–, glufosinate- and/or glyphosate-containing herbicides, or infested by insect pests that are susceptible to the Cry1Ac and/or Cry1F proteins.

The GMO Panel considers that the fitness advantage provided by the intended traits, and the observed reduction in 100-seed weight (see Section 3.5.5) will not allow the GM plant to overcome other biological and abiotic factors (described above) limiting plant's persistence and invasiveness. Therefore, the presence of the intended traits and other observed differences will not affect the persistence and invasiveness of the GM plant.

In conclusion, the GMO Panel considers it unlikely that soybean DAS–81419–2 × DAS–44406–6 will differ from conventional soybean hybrid 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 × DAS–44406–6 seeds.

3.7.2. Potential for gene transfer

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 feral plants originating from spilled seeds.

3.7.2.1. Plant-to-microorganism gene transfer

The probability and potential adverse effects of HGT of the recombinant DNA have been assessed in previous GMO Panel Scientific Opinions for the single events (Table 1). This assessment included consideration of homology-based recombination processes, as well as non-homologous end joining and microhomology-mediated end joining. Possible fitness advantages that the bacteria in the receiving environments would gain from acquiring recombinant DNA were considered. No concern as a result of an unlikely, but theoretically possible, HGT of the recombinant genes to bacteria in the gut of domesticated animals and humans fed GM material or other receiving environments was identified.

[27] Dossier: Part II – Section 5; additional information: 10/5/2019.

The updated bioinformatic analyses provided by the applicant for events DAS–81419–2 and DAS–44406–6 confirm the assessments provided in context of the single applications (EFSA GMO Panel, 2016, 2017b).

Synergistic effects of the recombinant genes, for instance due to combinations of recombinogenic sequences, which would cause an increase in the likelihood for HGT or a selective advantage were not identified.

Therefore, the GMO Panel concludes that the unlikely, but theoretically possible, horizontal transfer of recombinant genes from this two-event stack soybean to bacteria does not raise any environmental safety concern.

3.7.2.2. Plant-to plant-gene transfer

The potential for occasional feral soybean DAS–81419–2 × DAS–44406–6 plants originating from seed import spills to transfer recombinant DNA to sexually compatible plants and the environmental consequences of this transfer were considered.

For plant-to-plant gene transfer to occur, imported GM soybean seeds need to germinate and develop into plants in areas containing sympatric wild relatives and/or cultivated soybean with synchronous flowering and environmental conditions favouring cross-pollination. It must be noted that most soybean DAS–81419–2 × DAS–44406–6 seeds are processed in the countries of production or in ports of importation.

Vertical gene transfer from soybean (*G. max*) is limited to the species of the subgenus *Soja* to which *G. max* belongs to, as well as the wild relatives *G. soja* and *G. gracilis*. Although wild relatives exist elsewhere, no wild relatives of the subgenus *Soja* have been reported in Europe (Dorokhov et al., 2004; Lu, 2005). Therefore, vertical gene transfer from GM soybean is restricted to cultivated soybean (*G. max)*.

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), although 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 (Caviness, 1966; Gumisiriza and Rubaihayo, 1978; Kikuchi et al., 1993; Ahrent and Caviness, 1994; Ray et al., 2003; Lu, 2005).

The potential of spilled soybean seeds to establish, grow and produce pollen is extremely low and transient (see Section 3.7.1). Therefore, the likelihood/frequency of cross-pollination between occasional feral GM soybean plants resulting from seed spillage, and weedy or cultivated soybean plants is also considered extremely low. 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 for the reasons given in Section 3.7.1, even if exposed to the intended herbicides.

3.7.3. Interactions of the GM plant with target organisms

Taking the scope of application EFSA-GMO-NL-2016-132 into account (no cultivation), potential interactions of occasional feral soybean DAS–81419–2 × DAS–44406–6 plants arising from seed import spills with the target organism are not considered a relevant issue.

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

Given that environmental exposure of non-target organisms to spilled GM seeds or occasional feral GM soybean plants arising from spilled GM seeds is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM soybean, potential interactions of the GM plant with non-target organisms are not considered GMO Panel to raise any environmental safety concern. Interactions that may occur between the Cry proteins (as mentioned in Section 3.4.1) will not alter this conclusion.

3.7.5. Interactions with the abiotic environment and biogeochemical cycles

Given that environmental exposure to spilled seeds or occasional feral soybean DAS–81419–2 × DAS–44406–6 plants arising from seed import spills is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM soybean,

potential interactions with the abiotic environment and biogeochemical cycles are not considered by the GMO Panel to raise any environmental safety concern.

3.7.6. Conclusion of the environmental risk assessment

The GMO Panel concludes that it is unlikely that soybean DAS–81419–2 × DAS–44406–6 would differ from conventional soybean varieties in its ability to persist under European environmental conditions. Considering the scope of application EFSA-GMO-NL-2016-132, interactions of occasional feral soybean DAS–81419–2 × DAS–44406–6 plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from soybean DAS–81419–2 × DAS–44406–6 to bacteria does not indicate a safety concern. Therefore, considering the combined traits and their interactions, the outcome of the agronomic and phenotypic analysis and the routes and levels of exposure, the GMO Panel concludes that soybean DAS–81419–2 × DAS–44406–6 would not raise safety concerns in the event of accidental release of viable GM soybean seeds into the environment.

3.8. Post-market monitoring[28]

3.8.1. Post-market monitoring of GM food and feed

The GMO Panel concludes that soybean DAS–81419–2 × DAS–44406–6 does not represent a nutritional concern and is as safe as the conventional counterpart and commercial non-GM soybean reference varieties tested. No post-market monitoring (EFSA GMO Panel, 2011b) of food and feed is considered necessary.

3.8.2. Post-market environmental monitoring

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

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

As the ERA did not identify potential adverse environmental effects from soybean DAS–81419–2 × DAS–44406–6, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for soybean DAS–81419–2 × DAS–44406–6 includes: 1) the description of a monitoring approach involving operators (federations involved in import and processing), reporting to the applicant, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment; 2) a coordinating system established by EuropaBio for the collection of information recorded by the various operators; and 3) the review of relevant scientific publications retrieved from literature searches (Lecoq et al., 2007; Windels et al., 2009). The applicant proposes to submit a PMEM report on an annual basis and a final report at the end of the authorisation period.

The GMO Panel considers that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of soybean DAS–81419–2 × DAS–44406–6. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

In the context of annual PMEM reports, the applicant should improve future literature searches according to the GMO Panel recommendations given in Section 3.3.

3.8.3. Conclusion on post-market monitoring

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 × DAS–44406–6.

4. Overall conclusions

The GMO Panel was asked to carry out a scientific assessment of soybean DAS–81419–2 × DAS–44406–6 for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003.

[28] Dossier: Part II – Section 5.

No new information on the single soybean events DAS–81419–2 and DAS–44406–6 that would lead to a modification of the original conclusions on their safety were identified.

The molecular characterisation, the comparative analysis (agronomic, phenotypic and compositional characteristics) and the outcome of the toxicological and allergenicity assessments indicate that the combination of the single soybean events and of the newly expressed proteins in the two-event stack soybean does not give rise to food and feed safety and nutritional concerns. The GMO Panel concludes that the two-event stack soybean, as described in this application, is as safe as and nutritionally equivalent to the conventional counterpart and the non-GM reference varieties tested, and that no post-market monitoring of food and feed is considered necessary.

There is a very low likelihood of environmental effects resulting from the accidental release of viable seeds from soybean DAS–81419–2 × DAS–44406–6 into the environment. The PMEM plan and reporting intervals are in line with the intended uses of soybean DAS–81419–2 × DAS–44406–6.

Based on the relevant publications retrieved through systematic literature searches, the GMO Panel does not identify any safety issues pertaining to the intended uses of soybean DAS–81419–2 × DAS–44406–6. In the context of annual PMEM reports, the applicant could further fine-tune future literature searches according to the GMO Panel recommendations.

In addition, the GMO Panel considered the additional unpublished studies listed in Appendix A. This new information does not raise any concern for human and animal health and the environment regarding the two-event stack soybean.

In conclusion, the GMO Panel considers that soybean DAS–81419–2 × DAS–44406–6, as described in this application, is as safe as the conventional counterpart and the tested non-GM reference varieties with respect to potential effects on human and animal health and the environment.

Documentation as provided to EFSA

- Letter from the Netherlands to EFSA received on 02 March 2016 concerning a request for authorisation of genetically modified insect-resistant and herbicide-tolerant and soybean DAS-81419-2 × DAS-44406-6 for food and feed uses, import and processing submitted in accordance with Regulation (EC) No 1829/2003 by Dow Agrosciences LCC (EFSA-GMO-NL-2016-132).
- Application EFSA-GMO-NL-2016-132 validated by EFSA, 9 August 2016.
- Clock stops on 9 August 2016 due to single event not assessed (soybean DAS-44406-6 - Application EFSA-GMO-NL-2013-116).
- EFSA Request for supplementary information to the applicant on behalf of EURL-GMFF, 16 August 2016.
- EFSA starts Risk assessment, finalisation of single event (soybean DAS-44406-6 - Application EFSA-GMO-NL-2013-116), 28 February 2017.
- EFSA Request for supplementary information to the applicant, 7 March 2017.
- EFSA Request for supplementary information to the applicant, 20 April 2017.
- Information received from the applicant, 15 May 2017.
- Information received from the applicant, June 2017.
- EFSA Request for supplementary 26 information to the applicant, 28 June 2017.
- Information received from the applicant, 25 August 2017.
- EFSA Request for supplementary information to the applicant, 18 October 2017.
- EFSA Request for supplementary information to the applicant, 16 November 2017.
- Information received from the applicant, 13 December 2017.
- EFSA Request for supplementary information to the applicant, 21 December 2017.
- Information received from the applicant, 10 January 2018.
- EFSA Request for supplementary information to the applicant, 1 February 2018.
- Information received from the applicant, 28 February 2018.
- EFSA Request for supplementary information to the applicant, 21 March 2018.
- Information received from the applicant, 23 March 2018.
- Spontaneous information received from the applicant, 14 May 2018.
- EFSA Request for supplementary information to the applicant, 13 June 2018.
- EFSA Request for supplementary information to the applicant, 22 June 2018.
- Information received from the applicant, 27 August 2018.
- EFSA Request for supplementary information to the applicant, 13 September 2018.
- Information received from the applicant, 21 September 2018.

- EFSA Request for supplementary information to the applicant, 7 November 2018.
- Information received from the applicant, 18 December 2018.
- EFSA Request for supplementary information to the applicant, 19 December 2018.
- Information received from the applicant, 18 January 2019.
- EFSA Request for supplementary information to the applicant, 6 February 2019.
- Information received from the applicant, 12 February 2019.
- EFSA Request for supplementary information to the applicant, 13 February 2019.
- Information received from the applicant, 15 February 2019.
- EFSA Request for supplementary information to the applicant, 22 March 2019.
- Information received from the applicant, 2 May 2019.
- Spontaneous information received from the applicant, 10 May 2019.
- EFSA Request for supplementary information to the applicant, 7 June 2019.
- Information received from the applicant, 4 November 2019.
- EFSA Request for supplementary information to the applicant, 1 December 2019.
- EFSA Request for supplementary information to the applicant, 7 February 2020.
- Information received from the applicant, 8 April 2020.
- EFSA Request for supplementary information to the applicant, 12 May 2020.
- Information received from the applicant, 14 May 2020.
- EFSA Request for supplementary information to the applicant, 29 May 2020.
- Spontaneous information received from the applicant, 15 June 2020.
- Information received from the applicant, 15 June 2020.
- Spontaneous information received from the applicant, 10 August 2020.

References

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 twelve soybean cultivars in Arkansas. Crop Science, 34, 376–378. https://doi.org/10.2135/cropsci1994.0011183x003400020013x

Batista R, Martins I, Jenö P, Ricardo CP and Oliveira MM, 2007. A proteomic study to identify soya allergens – the human response to transgenic versus non-transgenic soya samples. International Archives of Allergy and Immunology, 144, 29–38. https://doi.org/10.1159/000102611

Cardineau GA, Stelman SJ and Narva KE, 2001. Plant-optimized genes encoding pesticidal toxins. US.

Caviness CE, 1966. Estimates of natural cross-pollination in Jackson soybeans in Arkansas. Crop Science, 6, 211–212. https://doi.org/10.2135/cropsci1966.0011183x000600020034x

Celesti-Grapow L, Pretto F, Carli E and Blasi C, eds, 2010. Flora vascolare alloctona e invasiva delle regioni d'Italia. Casa Editrice Università 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. Available online: http://www.fao.org/docrep/011/a1554e/a1554e00.htm

Dorokhov D, Ignatov A, Deineko E, Serjapin A, Ala A and Skryabin K, 2004. In: den Nijs HCM, Bartsch D and Sweet J, eds. Introgression from Genetically Modified Plants into Wild Relatives. CAB International, Amsterdam, the Netherlands. 151–161.

Duranti M and Gius C, 1997. Legume seeds: protein content and nutritional value. Field Crops Research, 53, 31–45. https://doi.org/10.1016/s0378-4290(97)00021-x

Eckes P, Vijtewaal B and Donn GJ, 1989. Synthetic gene confers resistance to the broad spectrum herbicide L-phosphinothricin in plants. Journal of Cellular Biochemistry, 41, 334. https://onlinelibrary.wiley.com/doi/10.1002/jcb.240410805

EFSA (European Food Safety Authority), 2010. Application of systematic review methodology to food and feed safety assessments to support decision making. EFSA Journal 2020;8(6):1637, 90 pp. https://doi.org/10.2903/j.efsa.2010.1637

EFSA (European Food Safety Authority), 2014. Explanatory statement for the applicability of the Guidance of the EFSA Scientific Committee on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed for GMO risk assessment. EFSA Journal 2014;12(10):3871, 25 pp. https://doi.org/10.2903/j.efsa.2014.3871

EFSA (European Food Safety Authority), 2015. Use of EFSA Comprehensive European Food Consumption Database for estimating dietary exposure to genetically modified foods. EFSA Journal 2015;13(2):4034, 11 pp. https://doi.org/10.2903/j.efsa.2015.4034

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), Mortensen A, Aguilar F, Crebelli R, Di Domenico A, Frutos MJ, Galtier P, Gott D, Gundert-Remy U, Lambre C, Leblanc J-C, Lindtner O, Moldeus P, Mosesso P, Oskarsson A, Parent-Massin D, Stankovic I, Waalkens-Berendsen I, Woutersen RA, Wright M, Younes M, Brimer L, Altieri A, Christodoulidou A, Lodi F and Dusemund B, 2017. Scientific opinion on the re-evaluation of lecithins (E 322) as a food additive. EFSA Journal 2017;15(4):4742, 74 pp. https://doi.org/10.2903/j.efsa.2017.4742

EFSA FEEDAP Panel (EFSA Panel on additives and products or substances used in animal feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos MdL, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J, Innocenti ML and Martino L, 2017. Guidance on the assessment of the safety of feed additives for the target species. EFSA Journal 2017;15(10):5021, 19 pp. https://doi.org/10.2903/j.efsa.2017.5021

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. https://doi.org/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. https://doi.org/10.2903/j.efsa.2010.1250

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010c. 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. https://doi.org/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. https://doi.org/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. https://doi.org/10.2903/j.efsa.2011.2316

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2015. Guidance on the agronomic and phenotypic characterisation of genetically modified plants. EFSA Journal 2015;13(6):4128, 44 pp. https://doi.org/10.2903/j.efsa.2015.4128

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. https://doi.org/10.2903/j.efsa.2016.4642

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

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, Messean A, Nielsen EE, Nogue F, Robaglia C, Rostoks N, Sweet J, Tebbe C, Visioli F, Wal J-M, Eigenmann P, Epstein M, Hoffmann-Sommergruber K, Koning F, Lovik M, Mills C, Moreno FJ, van Loveren H, Selb R and Fernandez Dumont A, 2017b. Guidance on allergenicity assessment of genetically modified plants. EFSA Journal 2017;15(5):4862, 49 pp. https://doi.org/10.2903/j.efsa.2017.4862

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition, and Allergies), 2010. Scientific Opinion on Dietary Reference Values for carbohydrates and dietary fibre. EFSA Journal 2010;8(3):1462, 77 pp. https://doi.org/10.2903/j.efsa.2010.1462

EFSA Scientific Committee, 2011. EFSA guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed. EFSA Journal 2011;9(12):2438, 21 pp. https://doi.org/10.2903/j.efsa.2011.2438

EFSA (European Food Safety Authority), Devos Y, Guajardo IM, Glanville J and Waigmann E, 2017a. Explanatory note on literature searching conducted in the context of GMO applications for (renewed) market authorisation and annual post-market environmental monitoring reports on GMOs authorised in the EU market. EFSA supporting publications 2017;14(4):EN-1207, 48 pp. https://doi.org/10.2903/sp.efsa.2017.en-1207

EFSA (European Food Safety Authority), Gennaro A, Gomes A, Herman L, Nogué F, Papadopoulou N and Tebbe C, 2017b. Technical report on the explanatory note on DNA sequence similarity searches in the context of the assessment of horizontal gene transfer from plants to microorganisms. EFSA supporting publications 2017;14(7):EN-1273, 11 pp. https://doi.org/10.2903/sp.efsa.2017.en-1273

EFSA (European Food Safety Authority), Dumont AF, Lanzoni A, Waigmann E and Paoletti C, 2018. Relevance of new scientific information (Santos-Vigil et al., 2018) in relation to the risk assessment of genetically modified crops with Cry1Ac. EFSA supporting publication 2018;15(11):EN-1504, 13 pp. https://doi.org/10.2903/sp.efsa.2018.en-1504

EFSA (European Food Safety Authority), Gomez Ruiz JA, Bresson J-L, Frenzel T and Paoletti C, 2019a. Statement on the human dietary exposure assessment to newly expressed proteins in GM foods. EFSA Journal 2019;17(7):5802, 18 pp. https://doi.org/10.2903/j.efsa.2019.5802

EFSA (European Food Safety Authority), Ardizzone M, Binaglia M, Cottrill B, Cugier J-P, Ferreira L, Gómez Ruiz JÁ, Innocenti M, Ioannidou S, López Puente S, Merten C, Nikolic M and Savoini G, 2019b. Scientific report on the animal dietary exposure: overview of current approaches used at EFSA. EFSA Journal 2019;17(11):5896, 18 pp. https://doi.org/10.2903/j.efsa.2019.5896

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. https://doi.org/10.1016/j.fct.2013.08.023

Gao Y, Fencil KJ, Xu X, Schwedler DA, Gilbert JR and Herman RA, 2006. Purification and characterization of a chimeric cry1f δ-endotoxin expressed in transgenic cotton plants. Journal of Agricultural and Food Chemistry, 54, 829–835. https://doi.org/10.1021/jf0523869

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.

Guzmán P, Saldaña B, Cámara L and Mateos GG, 2016. Influence of soybean protein source on growth performance and nutrient digestibility of piglets from 21 to 57 days of age. Animal Feed Science and Technology, 222, 75–86. https://doi.org/10.1016/j.anifeedsci.2016.10.004

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. https://doi.org/10.3109/10408444.2013.842956

Herrmann KM, 1995. The Shikimate pathway: early steps in the biosynthesis of aromatic compounds. Plant Cell, 7, 907–919. https://doi.org/10.1105/tpc.7.7.907

Honda K, Enoshima T, Oshikata T, Kamiya K, Hamamura M, Yamaguchi N, Nakamura K, Oguma Y, Fujiwara S, Takabe M, Sono A, Kawasaki T, Nasu M, Otsubo K and Wakigawa K, 2009. Toxicity studies of Asahi Kasei PI, purified phosphatidylinositol from soy lecithin. The Journal of Toxicological Sciences, 34, 265–280. https://doi.org/10.2131/jts.34.265

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. https://doi.org/10.3389/fpls.2015.00283

Ladics GS, Budziszewski GJ, Herman RA, Herouet-Guicheney C, Joshi S, Lipscomb EA, McClain S and Ward JM, 2014. Measurement of endogenous allergens in genetically modified soybeans – short communication. Regulatory Toxicology and Pharmacology, 70, 75–79. https://doi.org/10.1016/j.yrtph.2014.06.006

Lebrun M, Sailland A, Freyssinet M and Degryse E, 2003. Mutated 5-enolpyruvylshikimate-3-phosphate synthase, gene coding for said protein and transformed plants containing said gene. United States.

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. https://doi.org/10.1007/s00003-007-0244-1

L'Hocine L and Boye JI, 2007. Allergenicity of soybean: new developments in identification of allergenic proteins, cross-reactivities and hypoallergenization technologies. Critical Reviews in Food Science and Nutrition, 47, 2127–2143. https://doi.org/10.1080/10408390600626487

Liener IE, 1994. Implications of antinutritional components in soybean foods. Critical Reviews in Food Science & Nutrition, 34, 31–67. https://doi.org/10.1080/10408399409527649

Lu BR, 2005. Multidirectional gene flow among wild, weedy, and cultivated soybeans. In: Gressel J, ed. Crop Ferality and Volunteerism. OECD and Taylor & Francis, Florida, Boca Raton. pp. 137–147. https://doi.org/10.1201/9781420037999.ch9

Lu M, Jin Y, Cerny R, Ballmer-Weber B and Goodman RE, 2018. Combining 2-DE immunoblots and mass spectrometry to identify putative soybean (*Glycine max*) allergens. Food and Chemical Toxicology, 116, 207–215. https://doi.org/10.1016/j.fct.2018.04.032

Nasi A, Picariello G and Ferranti P, 2009. Proteomic approaches to study structure, functions and toxicity of legume seeds lectins. Perspectives for the assessment of food quality and safety. Journal of Proteomics, 72, 527–538. https://doi.org/10.1016/j.jprot.2009.02.001

OECD (Organisation for Economic Co-operation and Development), 1998. Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents, OECD Publishing, Paris. https://doi.org/10.1787/20745788

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.

OECD (Organisation for Economic Co-operation and Development), 2009. Guidance document on overview of residue chemistry studies. Series on Testing and Assessment number 64 and Series on Pesticides number 32, OECD Environment, Health and Safety Publications, Paris, ENV/JM/MONO (2009)31, 93 pp.
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. No. 25, 1–48.
OECD (Organisation for Economic Co-operation and Development), 2013. Guidance document on residues in livestock. Series on pesticides No. 73. Organisation for Economic Co-operation and Development, ENV/JM/MONO(2013)8, No. 25, 1–48. Paris.
OECD (Organisation for Economic Co-operation and Development), 2018. Advisory document of the Working Group on Good Laboratory Practice on the management, characterisation and use of test items. ENV/JM/MONO (2018) 6. No. 19, 1–20.
Ogawa T, Bando N, Tsuji H, Okajima H, Nishidawa K and Sasaoka K, 1991. Investigation of the IgE-Binding proteins in soybeans by immunoblotting with the sera of the Soybean-sensitive patients with atopic dermatitis. Journal of Nutritional Science and Vitaminology, 37, 555–565. https://doi.org/10.3177/jnsv.37.555
Owen MDK, 2005. Maize and soybeans – controllable volunteerism without ferality? In: Gressel J, ed. Crop ferality and volunteerism. OECD and Taylor & Francis, Boca Raton, Florida. pp. 149–165.
Parenti MD, Santoro A, Del Rio A and Franceschi C, 2019. Literature review in support of adjuvanticity/immunogenicity assessment of proteins. EFSA supporting publication 2019;EN-1551, 68 pp. https://doi.org/10.2903/sp.efsa.2019.en-1551
Ray JD, Kilen TC, Abel CA and Paris RL, 2003. Soybean natural cross-pollination rates under field conditions. Environmental Biosafety Research, 2, 133–138. https://doi.org/10.1051/ebr:2003005
Rougé P, Culerrier R, Granier C, Rancé F and Barre A, 2010. Characterization of IgE-binding epitopes of peanut (*Arachis hypogaea*) PNA lectin allergen cross-reacting with other structurally related legume lectins. Molecular Immunology, 47, 2359–2366. https://doi.org/10.1016/j.molimm.2010.05.006
Schnepf E, Crickmore N, Van Rie J, Lereclus D, Baum J, Feitelson J, Zeigler DR and Dean DH, 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiology and Molecular Biology Reviews, 62, 775–806. https://doi.org/10.1128/MMBR.62.3.775-806.1998
Selb R, Wal JM, Moreno FJ, Lovik M, Mills C, Hoffmann-Sommergruber K and Fernandez A, 2017. Assessment of endogenous allergenicity of genetically modified plants exemplified by soybean - where do we stand? Food and Chemical Toxicology, 101, 139–148. https://doi.org/10.1016/j.fct.2017.01.014
Shi L, Arntfield SD and Nickerson M, 2018. Changes in levels of phytic acid, lectins and oxalates during soaking and cooking of Canadian pulses. Food Research International, 107, 660–668. https://doi.org/10.1016/j.foodres.2018.02.056
Shibasaki M, Sumazaki R, Isoyama S and Takita H, 1992. Interaction of lectins with human IgE: IgE-binding property and histamine-releasing activity of twelve plant lectins. International Archives of Allergy and Immunology, 98, 18–25. https://doi.org/10.1159/000236160
Sys C, Van Ranst E, Debaveye J and Beernaert F, 1993. Land Evaluation. Part III: Crop requirements. Agricultural Publication No. 7. — Brussels, General Administration for Development Cooperation, 191 pp.
Thompson GA, Hiatt WR, Facciotti D, Stalker DM and Comai L, 1987. Expression in plants of a bacterial gene coding for glyphosate resistance. Weed Science, 35, 19–23. https://doi.org/10.1017/S0043174500060999
Wendel A, 1995. Lecithin. In: Kirk-Othmer Encyclopedia of chemical technology, Vol. 15. 4th Edition. Wiley-Interscience, New York. pp. 192–210. https://doi.org/10.1002/0471238961.1205030923051404.a01.pub2
Windels P, Alcalde E, Lecoq E, Legris G, Pleysier A, Tinland B and Wandelt C, 2009. General surveillance for import and processing: the EuropaBio approach. Journal of Consumer Protection and Food Safety, 3(S2), 14–16. https://doi.org/10.1007/s00003-009-0359-7
Wohlleben W, Arnold W, Broer I, Hillemann D, Strauch E and Pühler A, 1988. Nucleotide sequence of the phosphinothricin N-acetyltransferase gene from *Streptomyces viridochromogenes* Tü494 and its expression in *Nicotiana tabacum*. Gene, 70, 25–37. https://doi.org/10.1016/0378-1119(88)90101-1
Wright TR, Shan G, Walsh TA, Lira JM, Cui C, Song P, Zhuang M, Arnold NL, Lin G, Yau K, Russell SM, Cicchillo RM, Peterson MA, Simpson DM, Zhou N, Ponsamuel J and Zhang Z, 2010. Robust crop resistance to broadleaf and grass herbicides provided by aryloxyalkanoate dioxygenase transgenes. Proceedings of the National Academy of Sciences, 107, 20240–20245. https://doi.org/10.1073/pnas.1013154107
Wu G, 2014. Dietary requirements of synthesizable amino acids by animals: a paradigm shift in protein nutrition. Journal of Animal Science and Biotechnology, 5, 34. https://doi.org/10.1186/2049-1891-5-34
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. https://doi.org/10.1051/ebr:2007003

Abbreviations

2,4–D	2,4-dichlorophenoxyacetic acid
AAD–12	Aryloxyalkanoate dioxygenase

ADF	Acid detergent fibre
bw	Body weight
Bt	*Bacillus thuringiensis*
DNA	Deoxyribonucleic acid
dw	Dry weight
ELISA	Enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvyl-shikimate-3-phosphate synthase
ERA	Environmental risk assessment
FAO	Food and Agriculture Organization of the United Nations
GM	Genetically modified
GMO	Genetically modified organism
HGT	Horizontal gene transfer
IgE	Immunoglobulin E
ISO	International Organization for Standardization
NDF	Neutral detergent fibre
OECD	Organisation for economic co–operation and development
ORF	Open reading frames
PAT	Phosphinothricin acetyl transferase
PCR	Polymerase chain reaction
PMEM	Post-market environmental monitoring
SD	Standard deviation
UPLC-MS/MS	Ultra performance liquid chromatography – tandem mass spectrometer

Appendix A – List of additional unpublished studies performed by or on behalf of the applicant with regard to the evaluation of the safety of the food and feed for humans, animal and the environment for DAS–81419–2 × DAS–44406–6

Study identification	Title
170638	Bioinformatics evaluation of the putative reading frames across junctions and within the insert in DAS-44406-6 within the DAS-81419-2 × DAS-44406-6 soybean breeding stack for potential protein allergenicity and toxicity (2017)
170637	Assessment of the possibility of horizontal gene transfer from DAS-44406-6 within the DAS–81419–2 × DAS-44406-6 soybean breeding stack to microbial genomes (2017)
170636	Bioinformatics analysis of the flanking border sequences in DAS-44406-6 within the breeding stack DAS-81419-2 × DAS-44406-6 soybean (2017)
170635	Bioinformatics evaluation of the putative reading frames across junctions and within the insert in DAS-81419-2 within the DAS-81419-2 × DAS-44406-6 soybean breeding stack for potential protein allergenicity and toxicity (2017)
170634	Assessment of the possibility of horizontal gene transfer from DAS-81419-2 within the DAS–81419-2 × DAS-44406-6 soybean breeding stack to microbial genomes (2017)
170633	Bioinformatics analysis of the flanking border sequences in DAS-81419-2 within the breeding stack DAS-81419-2 × DAS-44406-6 soybean (2017)
160336	Nutrient composition of the soybean breeding stack DAS-81419-2 × DAS-44406-6 containing Cry1Ac, Cry1F, aryloxyalkanoate dioxygenase-12 (AAD-12), double mutant maize EPSPS (2mEPSPS), and phosphinothricin acetyltransferase (PAT) proteins
160335	Agronomic characteristics of the soybean breeding stack DAS-81419-2 × DAS-44406-6 containing Cry1Ac, Cry1F, aryloxyalkanoate dioxygenase-12 (AAD-12), double mutant maize EPSPS (2mEPSPS), and phosphinothricin acetyltransferase (PAT) proteins
160256	Statistical analysis of protein expression data from the combined trait product DAS-81419-2 × DAS–44406–6 soybean containing Cry1Ac, Cry1F, phosphinothricin acetyltransferase (PAT), aryloxyalkanoate dioxygenase (AAD-12) and double mutant maize EPSPS (2mEPSPS) proteins
140698	Quantitation of endogenous soybean allergens from soybean seed (DAS-81419-2 × DAS–44406-6) by liquid chromatography with tandem mass spectrometry (LC-MS/MS)
131109	Expressão de proteínas, composição nutricional e características agronômicas de uma cultivar de soja transformada contendo os eventos DAS-44406-6 e DAS-81419-2
131080[(a)]	DAS-81419-2 × DAS-44406-6 soybean: 90–day dietary toxicity study in Crl:CD(SD) rats
130585[(a)]	Broiler chicken feeding study using feeds containing DAS-81419-2 × DAS-44406-6 Soybean
120985	Molecular characterization of DAS-81419-2 × DAS-44406-6 soybean
120043.03	Field production and agronomic characteristics of the combined trait product DAS-81419-2 × DAS-44406–6 soybean containing Cry1Ac, Cry1F, aryloxyalkanoate dioxygenase-12 (AAD–12), double mutant maize EPSPS (2mEPSPS), and phosphinothricin acetyltransferase (PAT) proteins
120043.02	Protein Expression of the Combined Trait Product DAS-81419-2 × DAS-44406-6 Soybean Containing Cry1Ac, Cry1F, phosphinothricin acetyltransferase (PAT), aryloxyalkanoate dioxygenase (AAD-12) and double mutant maize EPSPS (2mEPSPS) proteins
120043.01	Nutrient composition of the combined trait product DAS-81419-2 × DAS-44406 soybean containing Cry1Ac, Cry1F, aryloxyalkanoate dioxygenase-12 (AAD-12), double mutant maize EPSPS (2mEPSPS), and phosphinothricin acetyltransferase (PAT) proteins

Appendix B – List of relevant publications identified by the applicant through systematic literature searches (2006 to May 2020)

Reference
De Cerqueira DT, Fast BJ, Silveira AC and Herman RA, 2019. Transgene-product expression levels in genetically engineered breeding stacks are equivalent to those of the single events. GM crops & food 10, 35–43.
Fast BJ, Shan G, Gampala SS and Herman RA, 2020. Transgene expression in sprayed and non- sprayed herbicide-tolerant genetically engineered crops is equivalent. Regulatory Toxicology and Pharmacology 111, 104572.
Gampala SS, Fast BJ, Richey KA, Gao Z, Hill R, Wulfkuhle B, Shan G, Bradfisch GA and Herman RA, 2017. Single-Event Transgene Product Levels Predict Levels in Genetically Modified Breeding Stacks. Journal of Agricultural and Food Chemistry 65, 7885–7892.
Hill RC, Fast BJ and Herman RA, 2017. Transgenesis affects endogenous soybean allergen levels less than traditional breeding. Regulatory Toxicology and Pharmacology 89, 70–73.

Appendix C – Protein expression data

Mean, standard deviation and range of protein levels (ng/mg dry weight) from soybean DAS-81419-2 × DAS-44406-6 (not treated) and DAS-81419-2, DAS-44406-6 (not treated), from field trials performed across nine locations in USA in 2012 (n = 36)

Protein	Event(s)	Leaf (V5)	Leaf (V10-12)	Forage (R3)	Root (R3)	Seeds (R8)
Cry1Ac	DAS-81419-2 × DAS-4406-6	8.68[(a)] ± 3.07 [(b)] (4.51–15.10)[(c)]	22.56 ± 8.16 (6.88–36.43)	4.24 ± 3.67 (0.25–15.74)	0.45 ± 0.36 (0.13–1.97)	1.50 ± 0.25 (0.93–1.90)
	DAS-81419-2	25.36 ± 8.49 (10.44–41.65)	23.74 ± 10.35 (6.86–37.84)	4.74 ± 2.89 (0.51–12.34)	0.72 ± 0.51 (0.20–2.90)	1.44 ± 0.28 (0.81–1.83)
Cry1F	DAS-81419-2 × DAS-4406-6	43.29 ± 17.61 (12.84–92.47)	42.59 ± 21.69 (6.82–89.50)	12.45 ± 19.66 (1.32–86.73)	2.88 ± 2.51 (0.47–11.46)	15.34 ± 2.88 (9.28–19.92)
	DAS-81419-2	36.71 ± 13.90 (10.29–61.01)	45.94 ± 19.91 (12.28–87.13)	11.26 ± 12.85 (1.28–68.34)	3.85 ± 3.19 (0.64–16.03)	16.58 ± 3.53 (8.04–22.46)
PAT	DAS-81419-2 × DAS-4406-6	16.95 ± 4.04 (8.13–24.23)	22.71 ± 3.07 (16.68–27.86)	6.30 ± 2.19 (0.76–9.39)	1.89 ± 0.28 (1.45–2.50)	2.44 ± 0.55 (0.76–3.52)
	DAS-81419-2	6.53 ± 1.87 (2.41–9.58)	8.22 ± 1.16 (6.25–11.01)	2.66 ± 0.74 (1.25–5.06)	0.94 ± 0.41 (0.72–3.25)	0.86 ± 0.14 (0.58–1.15)
	DAS-44406-6	11.56 ± 1.98 (6.36–16.19)	12.41 ± 2.65 (4.31–18.36)	3.71 ± 1.03 (1.42–5.28)	1.46 ± 0.35 (0.83–2.16)	1.61 ± 0.27 (0.95–2.12)
AAD-12	DAS-81419-2 × DAS-4406-6	50.91 ± 12.64 (24.90–81.53)	80.12 ± 22.5 (38.76–127.56)	38.46 ± 15.9 (10.66–66.36)	15.29 ± 3.88 (5.89–24.72)	23.98 ± 3.54 (17.50–33.32)
	DAS-44406-6	40.37 ± 16.71 (18.77–88.06)	92.06 ± 23.52 (52.37–131.80)	41.51 ± 13.08 (9.79–62.27)	16.39 ± 5.07 (7.18–29.59)	20.86 ± 4.19 (13.26–36.40)
2mEPSPS	DAS-81419-2 × DAS-4406-6	3,420.33 ± 1,567.64 (991.44-6,693.53)	4,645.40 ± 1,249.35 (1,812.45–7,106.37)	465.41 ± 199.91 (31.97–792.74)	186.13 ± 39.07 (108.62–285.45)	10.70 ± 10.13 (4.48–43.10)
	DAS-44406-6	4,253.17 ± 1,796.22 (1,141.78–8,766.48)	4,916.22 ± 1,755.02 (2,309.19–9,735.34)	454.66 ± 117.34 (178.40–657.43)	86.54±27.76 (36.80–164.80)	9.65 ± 7.68 (5.21–35.00)

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

Appendix D – Statistically significant findings in the 90-day toxicity study in rats on the whole food and feed from soybean DAS-81419-2 and DAS–44406–6

Study on DAS-81419-2

Statistically significant parameter/ endpoint	Finding	GMO Panel interpretation
Mean body weight gain	Decreased, low dose group	Not of toxicological relevance – Sporadic (day 43–50) – Did not impact on the overall BWG over the entire duration of the study – Did not impact on the mean body weight – Not dose-related (not seen in the high dose group)
Feed conversion efficiency	Increased in females, high dose	Not of toxicological relevance – Slight
Motor activity	Reduced in both treated groups during interval 5 and the overall activity was reduced in the high dose group	Not of toxicological relevance – This is a parameter with great variability and the values in the treated groups are within the normal background variation
Platelet count	Reduced count in the low dose group (5%)	Not of toxicological relevance –Small magnitude –Not dose related (not seen in the high dose group –Values are within the normal range for rats of this strain and age
Coagulation parameters (PT & APTT)	Increased in the low dose group (< 5%)	Not of toxicological relevance – Small magnitude Not dose related (not seen in the high dose group) – Values are within the normal range for rats of this strain and age.
ALT and AST	Reduced significantly in the low-dose groups and non-significantly in the high-dose groups (approximately 10%)	Not of toxicological relevance – An increase is indicative of liver damage but a reduction is not adverse in isolation. There were no pathological changes in the liver
Serum potassium	Increased in the high-dose groups (5%)	Not of toxicological relevance – Within the normal physiological range – No related changes in other electrolytes or findings of pathological changes to the kidney

Study on DAS-44406-6

Statistically significant parameter/ endpoint	Finding	GMO Panel interpretation
Body weight, body weight gain and food consumption	Reductions in the high dose groups, for a number of intervals throughout the study and overall (< 10%)	Not of toxicological relevance – Small magnitudes within normal variation for the strain and age of rat

Statistically significant parameter/ endpoint	Finding	GMO Panel interpretation
Prothrombin time	Increases in the low-dose group (< 5%)	Not of toxicological relevance – Small magnitude Within normal variation for the strain and age of rat – Not seen in the high-dose group (no dose relationship)
BUN	Reduced in the high-dose group, by (< 10%)	Not of toxicological relevance – BUN increase is indicative of kidney damage but a reduction is not adverse in isolation; – No pathological changes in the kidney.
ALT and AST	Reduced in test-diet given groups (approximately 10%)	Not of toxicological relevance – An increase of these enzymes is indicative of liver damage but a reduction is not adverse in isolation; – No pathological changes in the liver.
Motor activity counts	Reduced in the top dose animals overall and for two intervals	Not of toxicological relevance – Parameter with great variability – Values in the treated groups are within the normal background variation
Ovary weights (absolute and relative to brain and body weight)	Reduced in the low-dose group (< 10%)	Not of toxicological relevance – Small magnitude – Not seen in the high-dose group (no dose relationship) – No gross pathological findings in the low-dose group and no gross or histopathological findings in the ovaries of high dose animals (low-dose animals were not examined histopathologically)
Testes weights relative to brain weight	Decreased in the high-dose males (7%)	Not of toxicological relevance – Due primarily to one rat with a very low testes weight (< 50% of the mean). – The mean absolute testes weights and the testes weights relative to body weight were not statistically significantly different from the concurrent controls – Histopathological findings associated informing on the background condition of this finding