

Assessment of genetically modified soybean GMB151 × DAS-44406-6 (application GMFF-2024-21774, EFSA-Q-2024-00330)

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The declarations of interest of all scientific experts active in EFSA's work are available at <https://open.efsa.europa.eu/experts>.

Abstract

Genetically modified soybean GMB151 × DAS-44406-6 was developed by crossing to combine two single events: GMB151 and DAS-44406-6. The two-event stack soybean expresses the Cry14Ab-1, HPPD-4, PAT, AAD-12 and 2mEPSPS proteins to confer herbicide tolerance and resistance to plant parasitic nematodes. The GMO Panel previously assessed the two single soybean events and did not identify safety concerns. No new data on the single soybean events were identified that could lead to modification of the original conclusions on their safety. 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. The GMO Panel concludes that two-event stack soybean, as described in this application, is as safe as the comparator and non-GM reference soybean varieties tested and no post-market monitoring of food/feed is considered necessary. In the case of accidental release of GM two-event stack soybean material into the environment, this would not raise environmental safety concerns. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of soybean GMB151 × DAS-44406-6. Post-market monitoring of food/feed is not considered necessary. The GMO Panel concludes that two-event stack soybean is as safe as its comparator and the tested non-GM reference varieties with respect to potential effects on human and animal health and the environment.

KEYWORDS

2mEPSPS, AAD-12, Cry14Ab-1, DAS-44406-6, genetic engineering, GM, GMB151, HPPD-4, import and processing, PAT, soybean (*Glycine max*)

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SUMMARY

Following the submission of application EFSA-Q-2024-00330 under Regulation (EC) No 1829/2003 from BASF Agricultural Solutions Seed US LLC (referred to hereafter as 'the applicant'), the Panel on Genetically Modified Organisms of the European Food Safety Authority (referred to hereafter as 'GMO Panel') was asked to deliver a Scientific Opinion on the safety of genetically modified (GM) nematode resistant and herbicide tolerant soybean (*Glycine max* L.) GMB151 × DAS-44406-6 (Unique Identifier BCS-GM151-6 × DAS-44406-6) (referred to hereafter as 'two-event stack soybean'), according to Regulation (EU) No 503/2013. The scope of application EFSA-Q-2024-00330 is for import, processing, and food and feed uses within the European Union (EU) of soybean GMB151 × DAS-44406-6, and does not include cultivation in the EU. The two-event stack soybean was produced by crossing to combine two single soybean events: GMB151, expressing Cry14Ab-1, to confer resistance to plant parasitic nematodes and HPPD-4 providing tolerance to HPPD inhibitor based herbicides; DAS-44406-6 expressing PAT, providing tolerance to glufosinate-based herbicides, AAD-12 providing tolerance to 2,4-D based herbicides and 2mEPSPS providing tolerance to glyphosate based herbicides.

The GMO Panel evaluated the two-event stack soybean with reference to the scope and appropriate principles described in its applicable guidelines for the risk assessment of GM plants and the post-market environmental monitoring (PMEM). The GMO Panel considered the information submitted in application EFSA-Q-2024-00330/GMFF-2024-21774, additional information provided by the applicant during the risk assessment, the scientific comments submitted by the Member States and the relevant scientific literature. For application EFSA-Q-2024-00330/GMFF-2024-21774, previous assessments of the two single events (GMB151, DAS-44406-6), provided a basis for the assessment of the two-event stack soybean. No safety concerns were identified by the GMO Panel in the previous assessments. No safety issue concerning the two single soybean events was identified by the updated bioinformatics analyses, nor reported by the applicant since the publication of the previous GMO Panel scientific opinions. Therefore, 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 carried out, 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. Environmental impacts and PMEM plan were also evaluated. The molecular characterisation data establish that the events GMB151, DAS-44406-6 combined in the two-event stack soybean have retained their integrity. Protein expression analysis showed that the levels of the newly expressed proteins are similar in the two-event stack soybean and in the single events.

Considering the selection of test materials, the field trial sites and the associated management practices, as well as the agronomic-phenotypic characterisation as indicator of the overall field trial quality, the GMO Panel concludes that the field trials are appropriate to support the comparative analysis. The comparative analysis of agronomic and phenotypic characteristics and seeds and forage composition identified no differences between soybean GMB151 × DAS-44406-6 and the non-GM comparator (referred to hereafter as comparator) that required further assessment except for the changes in crop development. These changes were further assessed for food/feed safety and environmental impact and raised no concern. The molecular characterisation, the comparative analysis 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. The GMO Panel concludes that soybean GMB151 × DAS-44406-6, is as safe as the comparator and the selected commercial non-GM soybean reference varieties (referred to hereafter as non-GM reference varieties). 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 GMB151 × DAS-44406-6 would not raise safety concerns in the case of accidental release of GM soybean material, including viable seeds, into the environment.

Given the absence of safety concerns for foods and feeds from soybean GMB151 × DAS-44406-6, the GMO Panel considers that post-market monitoring of these products is not necessary. The PMEM plan and reporting intervals are in line with the intended uses of the two-event stack soybean.

The literature searches did not identify any relevant publications on soybean GMB151 × DAS-44406-6. Therefore, the GMO Panel does not identify any safety issue pertaining to the intended uses of two-event stack soybean.

The GMO Panel concludes that soybean GMB151 × DAS-44406-6, as described in this application, is as safe as the comparator and the selected non-GM reference varieties with respect to potential effects on human and animal health and the environment.

1 | INTRODUCTION

The scope of the application GMFF-2024-21774, EFSA-Q-2024-00330 is for food and feed uses, import and processing of the genetically modified (GM) nematode resistant and herbicide tolerant soybean GMB151 × DAS-44406-6 and does not include cultivation in the European Union (EU).

1.1 | Background

On 6 June 2024, the European Food Safety Authority (EFSA) received from the Competent Authority of Belgium application GMFF-2024-21774, EFSA-Q-2024-00330 for authorisation of soybean GMB151 × DAS-44406-6 (Unique Identifier BCS-GM151-6 × DAS-44406-6), submitted by BASF Agricultural Solutions Seed US LLC (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003.¹ Following receipt of application GMFF-2024-21774, EFSA-Q-2024-00330, EFSA informed EU Member States (MS) and the European Commission (EC), and made the application available to them. Simultaneously, EFSA published summary of the application.²

EFSA checked the application for compliance with the relevant requirements of Regulation (EC) No 1829/2003 and Regulation (EU) No 503/2013,³ with the EFSA guidance documents, and, when needed, asked the applicant to supplement the initial application. On 13 September 2024, EFSA declared the application valid.

From validity date, EFSA and the Panel on Genetically Modified Organisms of the European Food Safety Authority (referred to hereafter as 'GMO Panel') endeavoured to respect a time limit of 6 months to issue a scientific opinion on application GMFF-2024-21774, EFSA-Q-2024-00330. This time limit was extended whenever EFSA and/or the GMO Panel requested supplementary information from the applicant. Pursuant 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. 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.⁴ The EU Member States had 3 months to make their opinion known on application GMFF-2024-21774, EFSA-Q-2024-00330 as of its date of validity.

1.2 | Terms of Reference as provided by the requestor

In accordance with 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 GMB151 × DAS-44406-6 in the context of its scope as defined in application GMFF-2024-21774, EFSA-Q-2024-00330.

Following 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. In addition to the present scientific opinion, EFSA was also asked to report on the particulars listed under Articles 6(5) and 18(5) of Regulation (EC) No 1829/2003, but not to give an opinion on them because they pertain to risk management.⁵

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a confidential and a non-confidential version of the dossier following the 'EFSA guidelines for the submission of an application to comply with the specific provisions of Regulation (EU) No 503/2013,⁶ and of the 'Administrative guidance for the preparation of applications' (EFSA, 2021). In accordance with Art. 38 of the Regulation (EC)

¹Regulation (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.

²Available online: <https://open.efsa.europa.eu/dossier/GMFF-2024-21774>.

³Commission 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. OJ L157, 8.6.2013, p. 1–48.

⁴Directive 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.

⁵These particulars are available online at: <https://open.efsa.europa.eu/study-inventory/EFSA-Q-2024-00330>.

⁶Commission 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. OJ L157, 8.6.2013, p. 1–48.

No 178/2002⁷ and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation, the non-confidential version of the dossier has been published on OpenEFSA.⁸

Pursuant to Art. 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations,⁹ EFSA carried out a public consultation on the non-confidential version of the application from 21 February to 14 March 2025 for which no comments were received.

The GMO Panel based its scientific assessment of the two-event stack soybean on the valid application GMFF-2024-21774, EFSA-Q-2024-00330, additional information provided by the applicant during the risk assessment, relevant scientific comments submitted by EU MS and relevant peer-reviewed scientific publications.

As part of this comprehensive information package, the GMO Panel received an additional unpublished study submitted by the applicant in order to comply with the specific provisions of Regulation (EU) No 503/2013. This additional unpublished study is listed in [Appendix A](#).

2.2 | Methodologies

The GMO Panel conducted its assessment in line with the principles described in Regulation (EU) No 503/2013 and in the applicable guidelines (i.e. EFSA GMO Panel, 2010a, 2011a, 2011b, 2015a, 2015b; EFSA Scientific Committee, 2011) and explanatory notes and statements (i.e. EFSA, 2010a, 2014, 2017, 2019a, 2019b; EFSA GMO Panel, 2010b) for the risk assessment of GM plants.

For this application, the contractors performed preparatory work for the evaluation of the applicant's completeness and quality of the DNA sequencing information (OC/EFSA/GMO/2020/01), the updated bioinformatics analyses (OC/EFSA/GMO/2021/06) and the literature search (OC/EFSA/MESE/2022/03-01-SC17).

3 | ASSESSMENT

3.1 | Introduction

Application GMFF-2024-21774, EFSA-Q-2024-00330 covers the two-event stack soybean GMB151 × DAS-44406-6.

The two-event stack soybean was produced by crossing to combine two single soybean events: GMB151, expressing Cry14Ab-1, to confer resistance to plant parasitic nematodes and HPPD-4 providing tolerance to HPPD inhibitor based herbicides; DAS-44406-6 expressing PAT, providing tolerance to glufosinate-based herbicides, AAD-12 providing tolerance to 2,4-D based herbicides and 2mEPSPS providing tolerance to glyphosate based herbicides.

The two single events were assessed previously (see [Table 2](#)) and no safety concerns for human and animal health or environmental safety were identified.

TABLE 1 Single soybean events of soybean GMB151 × DAS-44406-6 previously assessed by the GMO Panel.

Events	Application or mandate	Reference
GMB151	AP153	EFSA GMO Panel (2021)
DAS-44406-6	AP106	EFSA GMO Panel (2017a)

3.2 | Updated information on single events

Since publication of the scientific opinions on the single soybean events by the GMO Panel (see [Table 1](#)), no safety issues concerning the two single events have been reported by the applicant.

Updated bioinformatics analysis for event GMB151 to identify possible interruption of endogenous genes confirmed previous results indicating that the insertion may have occurred in the 3' UTR of the predicted gene BON1-associated protein 1-like protein. The possible consequences were already assessed in the single application and no safety concerns were identified (EFSA GMO Panel, 2021).

Updated bioinformatics analysis for event DAS-44406-6 to identify possible interruption of endogenous genes confirmed previous results indicating the deletion of a 4 Kb genomic region at the insertion site. Nevertheless, no known endogenous genes have been deleted or interrupted, confirming the previous assessment (EFSA GMO Panel, 2017a).

Updated bioinformatics analyses of the amino acid sequence of the newly expressed proteins Cry14Ab-1, HPPD-4, PAT, AAD-12 and 2mEPSPS confirmed previous results indicating no significant similarities to known allergens. While the updated bioinformatics analyses to identify significant similarities to toxins for Cry14Ab-1, PAT, AAD-12 and 2mEPSPS proteins confirmed previous results indicating no significant similarities to known toxins, the updated analysis for HPPD-4 identified

⁷Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–48.

⁸<https://open.efsa.europa.eu/dossier/GMFF-2024-21774>.

⁹Decision available at: https://www.efsa.europa.eu/sites/default/files/corporate_publications/files/210111-PAs-pre-submission-phase-and-public-consultations.pdf.

a significant similarity to VLLY_VIBVU described in UniProt database as hemolysin VIIY from *Vibrio vulnificus* (strain CMCP6) which was not previously reported in the single event GMB151 (EFSA GMO Panel, 2021). The significance of this similarity and the consequences for the safety of event GMB151 expressing HPPD-4 are assessed in Section 3.4.3.1.

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 GMB151 and DAS-44406-6 confirmed previous analyses (Table 1). These analyses indicate that the production of new peptides showing significant similarities to toxins or allergens for any of the events in soybean GMB151 × DAS-44406-6 is highly unlikely.

In order to assess the possibility for horizontal gene transfer (HGT) by homologous recombination (HR), the applicant performed a sequence identity analysis with microbial DNA for soybean events GMB151 and DAS-44406-6 according to EFSA (2017). For event GMB151, no regions with significant homology were identified. For event DAS-44406-6, significant homology to multiple bacterial genomes were confirmed for the genetic elements *AtuORF23 3' UTR*, *AtuORF1 3' UTR* and T-DNA border-intervening sequence. The likelihood and potential consequences of plant-to-bacteria gene transfer are described in Section 3.4.4.2.

3.3 | Systematic literature review¹⁰

The GMO Panel assessed the applicant's literature searches on soybean GMB151 × DAS-44406-6, which include a scoping review, according to the guidelines given in EFSA (2010a, 2019b).

A systematic review as referred to in Regulation (EU) No 503/2013 has not been provided in support to the risk assessment of application GMFF-2024-21774, EFSA-Q-2024-00330. Based on the outcome of the scoping review, the GMO Panel agrees that there is limited value in undertaking a systematic review for soybean GMB151 × DAS-44406-6 at present.

The GMO Panel considered the overall quality of the performed literature searches acceptable. The literature searches did not identify any relevant publications on soybean GMB151 × DAS-44406-6 therefore the GMO Panel does not identify any safety issue pertaining to the intended uses of two-event stack soybean.

3.4 | Risk assessment of the two-event stack soybean GMB151 × DAS-44406-6

3.4.1 | Molecular characterisation¹¹

In line with the requirements laid down by Regulation (EU) 503/2013, the possible impact of the combination of the events on their integrity and the expression levels of the newly expressed proteins, as well as the possible impact of the combination of the biological functions conferred by each of the inserts, are considered below.

Genetic elements and biological function of the inserts.

Soybean events GMB151 and DAS-44406-6 were combined by conventional crossing to produce the two-event stack soybean GMB151 × DAS-44406-6. The structure of the inserts introduced into the two-event stack soybean is described in detail in the respective EFSA 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.

TABLE 2 The intended effects of the inserts in soybean GMB151 × DAS-44406-6 are summarised in Table 3. Based on the known biological functions of the newly expressed proteins (Table 3) no potential interactions are foreseen. Table 2 Genetic elements in the expression cassettes of the events stacked in soybean GMB151 × DAS-44406-6.

Event	Promoter	5' UTR	Transit peptide	Coding region	Terminator
GMB151	Pubi10At (<i>Arabidopsis thaliana</i>)	–	–	<i>cry14Ab-1.b^a</i> (<i>Bacillus thuringiensis</i>)	35S 3' UTR (Cauliflower Mosaic Virus)
	P2 × 35S (Cauliflower Mosaic Virus)	LteV leader sequence (Tobacco Etch Virus)	TPotpY-1Pf (<i>Zea mays</i> and <i>Helianthus annuus</i>)	<i>hppdPf-4Pa^a</i> (<i>Pseudomonas fluorescens</i>)	35S 3' UTR (Cauliflower Mosaic Virus)
DAS-44406-6 ^b	Histone H4A748 (<i>Arabidopsis thaliana</i>)	Histone 3 gene intron (<i>Arabidopsis thaliana</i>)	TPotp C (maize, sunflower)	2mepsps (<i>Zea mays</i>)	Histone H4A748 3' UTR

(Continues)

¹¹Dossier: Part II – Section 1.2; additional information: 5/11/2024, 10/12/2024, 13/3/2025, 28/5/2025, 22/7/2025, 13/9/2025 and 12/11/2025.

¹⁰Dossier: Part II – Section 7.0; additional information: 4/10/2024, 12/11/2025.

TABLE 2 (Continued)

Event	Promoter	5' UTR	Transit peptide	Coding region	Terminator
	<i>AtUbi10</i> (<i>Arabidopsis thaliana</i>)	<i>Ubi10</i> (<i>Arabidopsis thaliana</i>)	–	<i>aad-12^a</i> (<i>Delftia acidovorans</i>)	<i>AtuORF23</i> 3' UTR (<i>Agrobacterium tumefaciens</i>)
	<i>CsVMV</i> (Cassava vein mosaic virus)	<i>CsVMV</i> 5' UTR (Cassava vein mosaic virus)	–	<i>pat^a</i> (<i>Streptomyces viridochromogenes</i>)	<i>AtuORF1</i> 3' UTR (<i>Agrobacterium tumefaciens</i>)

Abbreviation: –, When no element was specifically introduced to optimise expression.

^aCodon optimised.

^bA matrix attachment region (MAR) of RB7 was added to the *2mepsps* cassette to facilitate expression.

TABLE 3 Characteristics and intended effects of the events stacked in soybean GMB151 × DAS-44406-6.

Event	Protein	Donor organism and biological function	Intended effects in GM plant
GMB151	Cry14Ab-1	Based on a gene from <i>Bacillus thuringiensis</i> . Cry14Ab-1 protein is a delta-endotoxin belonging to the 'nematicidal Branch' of Cry proteins	Event GMB151 expresses Cry14Ab-1 protein from <i>Bacillus thuringiensis</i> , conferring resistance to plant parasitic nematodes
	HPPD-4	Based on a gene from <i>Pseudomonas fluorescens</i> . HPPD enzyme is involved in aerobic tyrosine catabolism	Event GMB151 expresses the HPPD enzyme, which confers tolerance to HPPD inhibitor herbicides, such as isoxaflutole
DAS-44406-6	2mEPSPS	Based on a gene from <i>Zea mays</i> . 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) is an enzyme involved in the shikimic acid biosynthesis in plants	Event DAS-44406-06 expresses the 5-enolpyruvylshikimate-3-phosphate synthase protein from <i>Zea mays</i> with two amino acid substitutions, providing glyphosate tolerance
	AAD-12	Based on a gene from <i>Delftia acidovorans</i> . AAD-12 enzyme degrades 2,4-D into 2,4-dichlorophenol (DCP), which is no longer active as an herbicide	Event DAS-44406-06 expresses the aryloxyalkanoate dioxygenase-12 enzyme (AAD-12) from <i>Delftia acidovorans</i> , conferring tolerance to herbicides such as 2,4-D
	PAT	Based on a gene from <i>Streptomyces viridochromogenes</i> . Phosphinothricin-acetyl-transferase (PAT) enzyme acetylates L-glufosinate-ammonium	Event DAS-44406-06 expresses the PAT protein, which confers tolerance to glufosinate ammonium-based herbicides

3.4.1.1 | Integrity of the events in the two-event stack

The genetic stability of the inserted DNA over multiple generations in the single soybean events GMB151 and DAS-44406-6 was demonstrated previously (Table 1 and Section 3.2). The integrity of these events in soybean GMB151 × DAS-44406-6 was analysed by polymerase chain reaction (PCR) followed by NGS sequence analysis for GMB151 and by Sanger sequence analysis in the case of DAS-44406-6, demonstrating that the sequences of the events (inserts and their flanking regions) in the two-event soybean stack are identical to the sequences already assessed (Table 1 and Section 3.2), thus confirming that the integrity of these events was maintained in the two-event stack soybean.

3.4.1.2 | Information on the expression of the insert

In order to assess the changes in protein expression levels that 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.

Protein levels of the newly expressed proteins Cry14Ab-1, HPPD-4, PAT, AAD-12 and 2mEPSPS were analysed by an enzyme-linked immunosorbent assay (ELISA) in material harvested in field trials across four locations in the United States during the 2022 growing season. Samples analysed included leaf (BBCH 13–14, BBCH 16–17, BBCH 60–66, BBCH 71–79), root (BBCH 60–66, BBCH 71–79), flower (BBCH 60–66), forage (BBCH 71–79), whole plant (BBCH 81–89) and seed (BBCH 89–99) from plants treated with the intended herbicides.

The levels of all the newly expressed proteins in the two-event stack soybean and the corresponding singles were comparable in all tissues (Appendix B). Therefore, there is no indication of an interaction that may affect the levels of the newly expressed proteins in this stack.

3.4.1.3 | Conclusion on molecular characterisation

The molecular data establish that the events stacked in soybean GMB151 × DAS-44406-6 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins are similar in the two-event stack and in the respective single events. Therefore, there is no indication of an interaction that may affect the integrity of the events or the levels of the newly expressed proteins in this stack.

Based on the known biological functions (Table 3) of the newly expressed proteins, no potential functional interactions are foreseen.

3.4.2 | Comparative analysis¹²

3.4.2.1 | Overview of studies conducted for the comparative analysis

Application GMFF-2024-21,774 presents data on agronomic and phenotypic characteristics, as well as on forage and seed composition of the two-event stack soybean GMB151 × DAS-44406-6 (Table 4). In addition, the application contains data on characteristics of seed from the two-event stack soybean.

TABLE 4 Overview of the comparative analysis studies to characterise two-event stack soybean provided in the application GMFF-2024-21774, EFSA-Q-2024-00330.

Study focus	Study details	Comparator	Non-GM reference varieties
Agronomic and phenotypic analysis	Field study, US, 2022, 11 sites ^a	U15-606207	7 ^b
Compositional analysis	Field study, US, 2022, 8 sites ^a		

Abbreviation: GM, genetically modified.
^aEight field trials were used for both the compositional and the agronomic/phenotypic analysis: in USA, two sites in Indiana and Illinois, one site in Iowa, Nebraska, Pennsylvania and Missouri. Three field trials were used only for the agronomic/phenotypic analysis: two sites in Iowa and one site in Nebraska.
^bNon-GM soybean with their corresponding maturity group indicated in brackets were: Thorne (3.5); SA14-9653 (3.6); SA13-1385 (3.9); S13-10592C (4.5); E2692 (2.6); U14-910097 (2.9) and U16-932015(2.2).

3.4.2.2 | Experimental field trial design and statistical analysis

At each field trial site, the following materials were grown in a randomised complete block design with four replicates: the two-event soybean stack not exposed to the intended herbicides (not treated), the two-event soybean stack exposed to the intended herbicides (treated), the comparator U15-606207 and three non-GM reference varieties.

The agronomic, phenotypic and compositional data were analysed as specified by EFSA GMO Panel (2010b, 2011a). This includes, for each of the two treatments of the two-event soybean stack, the application of a difference test (between the GM soybean and the non-GM comparator) and an equivalence test (between the GM soybean and the set of non-GM reference varieties). The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).¹³

3.4.2.3 | Suitability of selected test materials

3.4.2.3.1 | Selection of the test materials

To obtain the stack soybean, the two events GMB151 and DAS-44406-6 were combined in the U15-606207 genetic background by conventional crossing and stabilised. This line was used in the comparative analysis.

The comparator used in the field trials is the non-GM soybean variety U15-606207, which has a similar genetic background as soybean GMB151 × DAS-44406-6 (as documented by the pedigree) and is considered to be a suitable comparator.

Two-event stack soybean and the comparator, both belong to maturity group 3.3 (as determined by the applicant), which is considered appropriate for growing in environments across USA, where the comparative field trials were conducted.

Commercial non-GM reference varieties with a maturity group ranging from 2.2 to 4.5 (as determined by the applicant) were selected and, at each site, three reference varieties were tested (see Table 4). On the basis of the provided information on relative maturity classes and year of commercialisation, the GMO Panel considers the selected non-GM reference varieties appropriate for the comparative assessment.

3.4.2.3.2 | Seed production and quality

Seeds of the two-event stack soybean and of the comparator used in the 2022 field trials were produced, harvested and stored under similar conditions, before being sown in the field trial sites. The seed lots were verified for their identity via event specific PCR analysis.

¹²Dossier: Part II – Section 1.3; additional information: 4/10/2024 and 10/12/2024.
¹³In 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).

Seed germination capacity of the two-event stack soybean was tested following the AOSA test protocol.¹⁴ Germination capacity of the two-event stack soybean was compared with its non-GM comparator and the results¹⁵ of these studies indicate that the seed germination of two-event stack soybean was not different.

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

3.4.2.3.3 | *Conclusion on suitability*

The GMO Panel is of the opinion that the two-event stack soybean, the comparator and the non-GM reference varieties were properly selected and are of adequate quality. Therefore, the test materials are considered suitable for the comparative analysis.

3.4.2.4 | *Representativeness of the receiving environments*

3.4.2.4.1 | *Selection of field trial sites*

The selected field trials sites were located in commercial soybean-growing regions of United States of America. The soil and climatic characteristics of the selected fields¹⁶ correspond to optimal, near optimal and sub-optimal conditions for soybean cultivation (Sys et al., 1993). The GMO Panel considers that the selected sites, including the subset chosen for the compositional analysis, reflect commercial soybean-growing regions in which the test materials are likely to be grown.

3.4.2.4.2 | *Meteorological conditions*

Maximum and minimum mean temperatures and sums of precipitations were provided for each site on a weekly basis. An exceptional weather condition was reported at one of the selected sites.¹⁷ However, due to the lack of major impacts on plant growth at this site, the GMO Panel considers that the exceptional weather condition did not invalidate the selection of the field trial sites for the comparative analysis.

3.4.2.4.3 | *Management practices*

The field trials included plots containing the two-event stack soybean, plots with the comparator and plots with non-GM reference varieties, mostly managed according to local agricultural practices. In addition, the field trials included plots containing the two-event stack soybean managed following the same agricultural practices, and exposed to two applications, one with the intended isoxaflutole (IFT) based herbicide at pre-emergence (BBCH 00–05), and the other with 2,4-D, glyphosate and glufosinate-based herbicides that were applied at BBCH 13–16 growth stage.¹⁸

At one field trial site,¹⁹ sowing occurred later than usual, resulting in a shifted growing cycle. The additional information indicated that the shifted growing cycle was unlikely to affect the representativeness of field trial conditions. The GMO Panel considers that the management practices, including sowing, harvesting and application of plant protection products were appropriate for the selected receiving environments.

3.4.2.4.4 | *Conclusion on representativeness*

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

3.4.2.5 | *Agronomic and phenotypic analysis*

Thirteen agronomic and phenotypic endpoints²⁰ plus information on abiotic stressors, disease incidence and arthropod damage were collected from the field trial sites (see Table 4). The endpoints lodging and seed loss were not analysed with formal statistical methods because of lack of variability in the data.

The statistical analysis (Section 3.4.2.2) was applied to 11 endpoints, with the following results:

¹⁴AOSA. 2013. Rules for testing seeds. Association of Official Seed Analysts.

¹⁵GM soybean GMB151 × DAS-44406-6 showed a mean germination of 95% while the non-GM comparator showed a mean of 96% under warm temperature conditions.

¹⁶Soil types of the field trials were clay loam, loam, sand, silt loam and silty clay loam; soil organic matter ranged from 0.9% to 4.6%; pH ranged from 5.6 to 7.4; average temperatures and sum of precipitations during the usual crop growing season ranged respectively from 17.2°C to 21.4°C and from 133 to 682 mm.

¹⁷Excessive rain was registered at one field trial in Indiana.

¹⁸BBCH scale describes phenological stages (Meier, 2001).

¹⁹The field trial located in Nebraska.

²⁰Early stand count, crop development, days to flowering, flowering duration, plant height, lodging, final stand count, days to maturity, seed loss, fruit count, seed moisture, seed weight and yield.

- For two-event stack soybean (not treated), the test of difference identified statistically significant differences with the comparator for eight endpoints.²¹ All these endpoints fell under equivalence category I.
- For two-event stack soybean (treated), the test of difference identified statistically significant differences with the comparator for 10 endpoints.²² All these endpoints fell under equivalence category I except for crop development, which fell under equivalence category IV.²³

Crop development for the two-event stack soybean (treated) was reduced with respect to the comparator and the non-GM reference varieties. However, as no impact on the other growth-related endpoints was observed, the GMO Panel considers that this result does not affect the use of the field trials for the comparative analysis. Crop development is assessed for potential environmental impact in Section 3.4.4.

3.4.2.6 | Compositional analysis

Forage and seeds of the two-event stack soybean harvested from eight sites (Table 4) were analysed for 114 constituents (nine in forage and 105 in seeds), including those recommended by OECD (OECD, 2012). The statistical analysis as described in Section 3.4.2.2 was not applied to 26 seed constituents because their concentration in more than one third of the samples were below the limit of quantification.²⁴

The statistical analysis was applied to a total of 88 constituents (79 in seeds²⁵ and 9 in forage²⁶); a summary of the outcome of the test of difference and the test of equivalence is presented in Table 5:

- For two-event stack soybean not treated with the intended herbicides, statistically significant differences with the non-GM comparator were found for 37 endpoints (2 in forage and 35 in seeds). All these endpoints fell under equivalence category I or II.
- For two-event stack soybean treated with the intended herbicides, statistically significant differences with the comparator were found for 53 endpoints (6 in forage and 47 in seeds). All these endpoints fell under equivalence category I or II.

TABLE 5 Outcome of the comparative compositional analysis in seeds and forage for two-event stack soybean. The table shows the number of endpoints in each category.

		Test of difference ^a			
		Treated ^c		Not treated ^c	
		Not different	Significantly different	Not different	Significantly different
Test of equivalence ^b	Category I/II	34	53 ^d	50	37 ^d
	Category III/IV	1 ^e	–	1 ^e	–
	Not categorised	–	–	–	–
	Total endpoints	88		88	

^aComparison between two-event stack soybean and its comparator.

^bFour 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.

^cTreated/not treated with the intended herbicides.

^dEndpoints with significant differences between two-event stack soybean and its conventional counterpart falling in equivalence category I–II (treated and not treated). For seeds, both treated and not treated: moisture, ash, crude protein, ADF, histidine, serine, threonine, tyrosine, valine, tryptophan, 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), calcium, magnesium, manganese, thiamine, pantothenic acid, phyloquinone, daidzein, total isoflavones, lectins, raffinose, gly m Bd 28k, δ-tocopherol, β-tocotrienol, total tocotrienols; only treated: crude fat, NDF, total dietary fibre, aspartic acid, glycine, leucine, phenylalanine, proline, potassium, α-tocopherol, phytic acid, gly m 6, gly m 8; only not treated: alanine. For forage, both treated and not treated: moisture; only treated: ash, carbohydrates, crude protein, NDF, calcium; only not treated: ADF.

^eEndpoint in seeds with no significant differences between two-event stack soybean and its comparator and falling under equivalence category III: stachyose.

²¹Early stand count, crop development, days to flowering, flowering duration, plant height, final stand count, seed moisture and seed weight.

²²Early stand count, crop development, days to flowering, flowering duration, plant height, final stand count, days to maturity, seed moisture, seed weight and yield.

²³For crop development (%), the estimated mean values for the comparator, the GM soybean (untreated), the GM soybean (treated) and the reference varieties were 67.0, 60.7, 51.5 and 67.6, respectively; the equivalence limits were (55.5, 79.6).

²⁴Caprylic 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), stearidonic acid (C18:4n-3), eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3n-3), dihomo-γ-linolenic acid (C20:3n-6), bishomostearidonic acid (C20:4n-3), arachidonic acid (C20:4n-6), eicosapentaenoic acid (C20:5), erucic acid (C22:1), clupanodonic acid (C22:5n-3), osbond acid (C22:5n-6), docosahexaenoic acid (C22:6), sodium, homogentisic acid, β-carotene, α-tocotrienol, γ-tocotrienol, δ-tocotrienol.

²⁵Seed constituents included proximates and fibre content (ash, carbohydrates, moisture, crude protein, crude fat, acid detergent fibre (ADF), neutral detergent fibre (NDF), total dietary fibre), minerals (calcium, phosphorus, copper, iron, magnesium, manganese, potassium, zinc), amino acids (alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine), fatty acids (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), lignoceric acid (C24:0)), vitamins (α-tocopherol, thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, folic acid, and phyloquinone), tyrosine pathway metabolites (β-tocopherol, γ-tocopherol, δ-tocopherol, total-tocopherols, β-tocotrienol, total-tocotrienols), isoflavones (daidzein, genistein, glycitein and total isoflavones), endogenous allergens (gly m 1, gly m 3, gly m 4, gly m 5 (β-conglycinin), gly m 6 (glycinin), gly m 7, gly m 8, gly m Bd 28k, gly m Bd 30k, Kunitz trypsin inhibitor 1 and Kunitz trypsin inhibitor 3), other compounds (phytic acid, raffinose, lectins, stachyose, trypsin inhibitor).

²⁶Forage constituents included ash, carbohydrates, moisture, crude protein, crude fat, acid detergent fibre (ADF), neutral detergent fibre (NDF), calcium and phosphorus.

The GMO Panel assessed all the significant differences between the two-event stack soybean and the comparator, taking into account potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties. No endpoints were identified that showed significant differences between the two-event stack soybean and the comparator and fell under category III/IV.

Metabolites closely related to the tyrosine catabolic pathway (Kramer et al., 2014) were included among the constituents analysed in the two-event stack soybean, i.e. α -tocopherol, β -tocopherol, γ -tocopherol, δ -tocopherol (and their corresponding tocotrienols), β -carotene, phylloquinone and homogentisic acid. As described above, the outcome of the comparative assessment indicated that no further assessment regarding food and feed safety is required for these compounds.

3.4.2.7 | Conclusion on comparative analysis

Considering the selection of test materials, the field trial sites and the associated management practices, as well as the agronomic-phenotypic characterisation as indicator of the overall field trial quality, the GMO Panel concludes that the field trials are appropriate to support the comparative analysis.

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

- None of the differences identified in agronomic and phenotypic characteristics tested between the two-event soybean stack and the comparator needs further assessment except for crop development, which is further assessed for potential environmental impact in Section 3.4.4.
- None of the differences identified in forage and seed composition between the two-event stack soybean and the comparator needs further assessment regarding food and feed safety.

3.4.3 | Food/feed safety assessment²⁷

The two single events included in this stack soybean application have been previously assessed and no safety concerns were identified by the EFSA GMO Panel (Table 1). The two-event stack soybean will undergo existing production processes used for conventional soybean. No novel production process is envisaged. Based on the outcome of the comparative assessment, processing of the two-event stack soybean into food and feed products is not expected to result in products differing from those of conventional non-GM soybean varieties currently in the EU market.²⁸

In accordance with Regulation (EU) No 503/2013 for the risk assessment of genetically modified food or feed containing stacked events, the GMO Panel evaluated potential interactions resulting from the combination of the single transformation events impacting on toxicity (Section 3.4.3.1), allergenicity (Section 3.4.3.2) and nutritional assessment (Section 3.4.3.3) described below.

3.4.3.1 | Toxicology

3.4.3.1.1 | Testing of NEPs

Five newly expressed proteins (Cry14Ab-1, HPPD-4, PAT, AAD-12 and 2mEPSPS) are expressed in the two-event stack soybean (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, or farmed and companion animals. The GMO Panel is not aware of any new information that would change its previous conclusions on the safety of these proteins. The potential for a functional interaction among the NEPs in the two-event stack soybean has been assessed by the applicant and evaluated by the GMO Panel with regard to human and animal health.

The updated bioinformatics analysis of the HPPD-4 amino acid sequence against known toxins showed significant identity to a protein of bacterial origin, which is annotated as a possible haemolysin (see Section 3.2). The HPPD-4 protein was tested for haemolytic potential *in vitro* and the protein, incubated with whole blood, was shown not to induce haemolysis. Some limitations in the study design were identified, mainly related to inconsistent performance of the positive control used. Taking into account: (i) previous assessments indicating the HPPD-4 and other highly similar proteins posed no safety concerns (EFSA GMO Panel, 2015b, 2021); (ii) the overall weight-of-evidence provided in the context of the GMB151 single application to support HPPD-4 safety (EFSA GMO Panel, 2021); and (iii) database issues suggesting that the annotation of the VLLY_VIBVU protein as a haemolysin is likely erroneous, as conserved domain analysis (InterPro) shows it contains only HPPD-related domains and no haemolysin-associated features, the GMO Panel identified no safety concerns associated with this bioinformatic hit and confirms the safety of HPPD-4 in soybean GMB151.

On the basis of the known biological function of the individual NEPs, there is currently no expectation for their possible interactions relevant to the food and feed safety of this two-event stack soybean (Table 6).

²⁷Dossier: Part II – Section 1.4, 1.5, 1.6, 2, 4, 7; additional information 13/3/2025, 22/7/2025, 13/9/2025, 12/11/2025.

²⁸On-going assessment of novel food applications (https://food.ec.europa.eu/food-safety/novel-food/authorisations/summary-applications-and-notifications_en).

TABLE 6 Intended effects and modes of action of NEPs in GMB151 × DAS-44406-6 soybean.

Protein	Intended effect and mode of action in GM plant
Cry14Ab-1	The Cry14Ab-1 protein confers resistance to plant parasitic nematodes by causing disruption of the midgut epithelium via pore formation.
HPPD-4	The HPPD-4 protein confers tolerance to HPPD-inhibiting herbicides, such as isoxaflutole, by modifying 4-hydroxyphenylpyruvate dioxygenase, an enzyme involved in the biosynthesis of homogentisate.
PAT	The PAT protein confers tolerance to glufosinate ammonium-based herbicides by acetylating L-phosphinothricin, the active isomer of the glufosinate-ammonium herbicide.
AAD-12	The AAD-12 protein confers tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D), facilitating the breakdown of these compounds into carbon sources for the bacterium (Wright et al., 2009).
2mEPSPS	The 2mEPSPS protein confers tolerance to glyphosate-based herbicides, acting on the shikimic acid pathway for the biosynthesis of aromatic amino acids in plants, showing high substrate specificity.

The GMO Panel concludes that there are no safety concerns to human and animal health related to the proteins Cry14Ab-1, HPPD-4, PAT, AAD-12 and 2mEPSPS in the two-event stack soybean.

3.4.3.1.2 | *Testing of new constituents other than proteins*

Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, no new constituents other than the NEPs have been identified in seed and forage from the two-event stack soybean. Therefore, no further food/feed safety assessment of components other than the NEPs is required.

3.4.3.1.3 | *Information on altered levels of food and feed constituents*

Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, none of the differences identified between the two-event stack soybean and its non-GM comparator in seed and forage composition require further assessment.

3.4.3.1.4 | *Testing of the whole genetically modified food and feed*

The GMO Panel considers animal studies with food/feed derived from the two-event stack soybean unnecessary based on the outcome of molecular characterisation, comparative analysis and toxicological assessment (EFSA GMO Panel, 2011a). There were no concerns regarding the stability and expression of the inserts or interaction between the transformation events, and no changes of toxicological concern in the composition of the two-event stack soybean were identified (Sections 3.4.1, 3.4.2.6, 3.4.3.1).

The GMO Panel had previously concluded that the 90-day feeding studies in rodents on whole food/feed from soybean GMB151 and DAS-44406-6 are in line with Regulation (EU) No 503/2013 and do not show adverse effects related to diets incorporating the respective single events (EFSA GMO Panel, 2020, 2021).

3.4.3.2 | *Allergenicity*

The strategies to assess the potential risk of allergenicity focus: (i) on the source of the recombinant protein; (ii) on the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons; and (iii) on whether the transformation may have altered the allergenic properties of the modified plant. Furthermore, the assessment also takes into account potential adjuvant properties of the newly expressed proteins, which is defined as the ability to enhance an allergic reaction.

3.4.3.2.1 | *Assessment of allergenicity of the newly expressed proteins*

The GMO Panel has previously evaluated the safety of the Cry14Ab-1, HPPD-4, PAT, AAD-12 and 2mEPSPS proteins individually, and no evidence of allergenicity was identified in the context of the applications assessed (Table 1). 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 simultaneous presence in this two-event stack soybean.

The GMO Panel has previously evaluated the safety of the newly expressed proteins, and no evidence of adjuvanticity were identified in the context of the applications assessed (Table 1). The GMO Panel did not find indications that the newly expressed proteins at the levels expressed in this two-event stack soybean might be adjuvants able to enhance an allergic reaction.

The applicant also provided information on the safety of the Cry14Ab-1, HPPD-4, PAT, AAD-12 and 2mEPSPS proteins regarding their potential to cause a celiac disease response. For such assessment, the applicant followed the principles

described in the EFSA GMO Panel guidance document (EFSA GMO Panel, 2017b). No indications of safety concern were identified by the GMO Panel as the relevant peptides containing the motif fail to mimic gluten sequences.

The GMO Panel considers that there are no indications that the newly expressed Cry14Ab-1, HPPD-4, PAT, AAD-12 and/or 2mEPSPS proteins in this two-event stack soybean may be allergenic.

3.4.3.2 | Assessment of allergenicity of the whole GM plant or crop

Soybean is considered a common allergenic food²⁹ (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 3.4.2) quantified using liquid chromatography with tandem MS, which has been previously considered acceptable (EFSA GMO Panel, 2010c, 2017b; Fernandez et al., 2013; Selb et al., 2017). These allergens were selected based on the list of potential soybean allergens described in the pertinent OECD document (OECD, 2012) and a scientific rationale 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 were identified by 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 soybean GMB151 × DAS-44406-6 when compared with that of the comparator and the non-GM reference varieties tested.

3.4.3.3 | Dietary exposure assessment to new constituents

In line with Regulation (EU) No 503/2013 the applicant provided dietary exposure estimates to Cry14Ab-1, HPPD-4, PAT, AAD-12 and 2mEPSPS proteins newly expressed in soybean GMB151 × DAS-44406-6. Dietary exposure was estimated based on protein expression levels reported in this application for soybean GMB151 × DAS-44406-6 treated with the intended herbicides (IFT-, 2,4-D, glyphosate- and glufosinate-based herbicides), the currently available consumption data and feed practices, the foods and feeds currently available on the market and the described processing conditions.

For the purpose of estimating dietary exposure, the levels of the NEPs in soybean GMB151 × DAS-44406-6 seeds, flowers³⁰ and forage were derived from material harvested in a field trial across four locations in the United States during the 2022 growing season (Table 7).

TABLE 7 Mean values (n = 12, µg/g dry weight and µg/g fresh weight) for newly expressed proteins in grains, forage and flowers from soybean GMB151 × DAS-44406-6 treated with the intended herbicides.^a

Protein	Tissue/developmental stage		
	Seeds/BBCH 89–99 (µg/g dry weight/µg/g fresh weight)	Forage/BBCH 71–79 (µg/g dry weight)	Flowers/BBCH 60–66 (µg/g dry weight) ^b
Cry14Ab-1 ^d	103.4/97.3	58.9	41.7
HPPD-4 ^d	12.7/11.9	16.3	29.5
2m EPSPS	3.6/3.4 ^c	71.0	128.5
PAT	2.8/2.6	6.0	7.0
AAD-12	66.7/62.3	69.2	66.1

^aIntended herbicides: HPPD inhibitor based herbicides, glufosinate-based herbicides, 2,4-D based herbicides, glyphosate based herbicides.

^bFlowers were used as surrogates of pollen for the analysis of the NEPs in soybean GMB151 × DAS-44406-6. The reported values in dry weight were expressed in 6% moisture content to account for standard moisture content of pollen supplements.

^cMean values for 2mEPSPS were estimated by using the LOQ (3.52 µg/g dw and 3.19 µg/g fw) in samples identified as below the LOQ. This approach is based on the substitution method used to treat left-censored data (EFSA, 2010b; WHO, 2009). For all samples reported as < LOD or as < LOQ, lower bound (LB) and upper bound (UB) values are derived by assigning a value of zero and LOD/LOQ, respectively. For the dietary exposure estimations, only the UB scenario (worst-case) is used; uncertainties linked to this approach will be considered should the exposure estimations be used in the safety assessment of the NEPs. This approach is also mentioned in the corresponding documents on animal and human dietary exposure (EFSA, 2019; EFSA GMO Panel, 2023).

^dValues as provided in ADR7 after extraction efficiency recalculation.

3.4.3.3.1 | Human dietary exposure

Chronic and acute estimations of dietary exposure to Cry14Ab-1, HPPD-4, PAT, AAD-12 and 2mEPSPS proteins newly expressed in soybean GMB151 × DAS-44406-6 were provided. The applicant followed the methodology described in the

²⁹Regulation (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.

³⁰Flowers (growth stage BBCH 60–66) were used as surrogates of pollen for the analysis of the NEPs in soybean GMB151 × DAS-44406-6.

EFSA Statement 'Human dietary exposure assessment to newly expressed protein in GM foods' to anticipate human dietary exposure making use of summary statistics of consumption (EFSA, 2019).

Human dietary exposure was estimated across European countries for different population groups: young population (infants, toddlers, 'other children'), adolescents, adult population (adults, elderly and very elderly) and special populations (pregnant and lactating women). Since no specific consumption data were available on commodities containing, consisting of or obtained from soybean GMB151 × DAS-44406-6 seeds, a conservative scenario with 100% replacement of conventional soybean by the GM soybean was considered. Consumption figures for all relevant commodities (e.g. soya bread, textured soy protein, soya drink, tofu, etc.) were retrieved from the EFSA Comprehensive European Food Consumption Database (EFSA consumption database).³¹

Mean protein expression values on fresh weight basis are considered as the most adequate to estimate human dietary exposure (both acute and chronic) when working with raw primary commodities that are commonly consumed as processed blended commodities (EFSA, 2019). Different recipes and factors were considered to estimate the amount of soybean seeds in the consumed commodities before assigning NEP levels to the relevant commodities.³² No losses in the NEPs during processing were considered.

The highest anticipated acute dietary exposure (highly exposed population) for the five NEPs assessed was in the age class 'Infants' with estimates that ranged from 36.4 mg/kg bw per day for PAT to 1336 mg/kg bw per day for Cry14Ab-1. The main contributor to the exposure in the dietary survey with the highest estimates would be 'Follow-on formula, soya-based, powder'. In the dietary exposure scenario anticipating acute consumption of soybean-derived protein supplements, the highest estimates would range between 45.7 mg/kg bw per day for PAT and 1680 mg/kg bw per day for Cry14Ab-1 (in the adult population, high consumers).

The highest anticipated chronic dietary exposure (highly exposed population) for the five NEPs assessed was in the age class 'Infants' with estimates that ranged from 12.2 mg/kg bw per day for PAT to 449 mg/kg bw per day for Cry14Ab-1. The main contributor to the exposure in the dietary survey with the highest estimates would be 'Follow-on formula, soya-based, powder'. In the dietary exposure scenario anticipating chronic consumption of soybean-derived protein supplements, the highest estimates would range between 29.7 mg/kg bw per day for PAT and 1092 mg/kg bw per day for Cry14Ab-1 (in the adult population, high consumers).

The GMO Panel considers pollen supplements as a possible contributor to the dietary exposure to Cry14Ab-1, HPPD-4, PAT, AAD-12 and 2mEPSPS, under the assumption that these supplements might be made of pollen from soybean GMB151 × DAS-44406-6. Due to the technical challenges related to the collection of pollen from soybean flowers (e.g. close flowers, low amounts produced), the applicant analysed the presence of the newly expressed proteins in flowers as pollen surrogates (see Table 7). The GMO Panel acknowledges the substantial uncertainty that the use of flowers as surrogates of pollen adds to the expression levels and, therefore, to the dietary exposure assessment. However, in the absence of identified hazards in the newly expressed proteins, these expression levels are accepted as surrogates.

Consumption data on pollen supplements are available for few consumers across seven different European countries.³¹ The low number of consumers available adds uncertainty to the exposure estimations which should be interpreted with care, and only allows the estimation of dietary exposure for average consumers. The highest mean acute dietary exposure for the five NEPs assessed would range 4.9 µg/kg bw per day for PAT and 90.1 µg/kg bw per day for 2mEPSPS proteins, in the elderly population. Similarly, the highest mean chronic dietary exposure in consumers of pollen supplements would be between 3.3 µg/kg bw per day for TDO and 60.0 µg/kg bw per day for DMO proteins, also in the elderly population.

3.4.3.3.2 | Animal dietary exposure

Anticipated dietary exposure to Cry14Ab-1, HPPD-4, PAT, AAD-12 and 2mEPSPS proteins in the two-event stack soybean was estimated by the applicant across farmed and companion animals, assuming the consumption of soybean products commonly entering the feed supply chain, as below described.

Estimations were based on the recommendations provided in EFSA GMO Panel, 2023.

A conservative scenario with 100% replacement of conventional soybean products by the two-event stack soybean products was considered.

Mean levels (dry weight) of the newly expressed proteins in full fat seed and forage/silage from the two-event stack soybean used for animal dietary exposure are listed in Table 7.

Mean levels (dry weight) of the newly expressed proteins in meal, hull and protein concentrate were calculated to be respectively 1.32, 0.34 and 2.07-fold higher than in seed, based on conversion factors that take into account the protein content in these feed materials relative to soybean seed, and assuming that no protein is lost during their production or processing. Values are reported in tables of Appendix C.

Some seed samples analysed in the two-event stack soybean for the presence of 2mEPSPS protein were below the limit of quantification (LOQ = 3.52 µg/g dry weight); for the purpose of estimating daily dietary intake (DDE), the determined values of LOQ were used as substitutes to compute an average concentration of 2mEPSPS in seed to be used for the exposure calculations.

³¹<https://www.efsa.europa.eu/en/applications/gmo/tools>. From version updated in March 2022.

³²Example: 100 grams of tofu contains approximately 26 g of soybean seeds. This results in 26.9 µg of Cry14Ab-1 per gram of tofu as compared to the 103.4 µg/g reported as Cry14Ab-1 mean concentration in the treated soybean seeds (see Table 7).

The applicant estimated dietary exposure in ruminants (dairy cow, beef cattle, dairy sheep and dairy goat) and other herbivorous animals (rabbit and horse), in pigs (fattening pig, lactating sow, piglet), in poultry (broiler, laying hens, turkey), in fish (salmon, carp) and in companion animals (cat and dog).

The dietary exposure was estimated via the consumption of soybean forage/silage, full fat seeds, meal, hulls and/or protein concentrate in standard diets or rations.

Estimated dietary exposure to Cry14Ab-1, HPPD-4, PAT, AAD-12 and 2mEPSPS proteins in the concerned animals is reported in [Appendix C](#).

3.4.3.4 | *Nutritional assessment of endogenous constituents*

The intended traits of this two-event stack soybean are nematode resistance and herbicide tolerance, with no intention to alter nutritional parameters. Comparison of the composition of this two-event stack soybean with the non-GM comparator and the commercial non-GM reference varieties did not identify differences that would require further safety assessment. From these data, the GMO Panel concludes that this two-event stack soybean is nutritionally equivalent to the comparator and non-GM reference varieties used.

3.4.3.5 | *Conclusions on the food/feed safety assessment*

The NEPs Cry14Ab-1, HPPD-4, PAT, AAD-12 and 2mEPSPS in the two-event stack soybean do not raise safety concerns for human and animal health. No interactions between NEPs relevant for food and feed safety were identified, and no overall toxicological concerns on the two-event stack soybean were identified. Similarly, the GMO Panel did not identify indications of safety concerns regarding allergenicity or adjuvant activity related to the presence of the NEPs in this two-event stack soybean, or regarding its overall allergenicity. Based on the outcome of the comparative assessment and the nutritional assessment, the GMO Panel concludes that the consumption of soybean GMB151 × DAS-44406-6 does not represent any nutritional concern, in the context of the scope of this application. The GMO Panel concludes that soybean GMB151 × DAS-44406-6, as described in this application, is as safe as the comparator and the non-GM reference varieties tested and no post-market monitoring of food/feed is considered necessary.

3.4.4 | Environmental risk assessment³³

Considering the scope of this application, which excludes cultivation, the environmental risk assessment (ERA) mainly takes into account: (i) the exposure of microorganisms to recombinant DNA in the gastrointestinal tract of animals fed with GM material and of microorganisms present in environments exposed to manure and faeces of these animals; and (ii) the deliberate or accidental release of GM material into the environment, including spillage of viable two-event stack soybean seeds during transportation and/or processing (EFSA GMO Panel, [2010a](#)).

3.4.4.1 | *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, absence of a dormancy phase and susceptibility to plant pathogens (OECD, [2000](#)). Additionally, soybean is a sub-tropical species susceptible to cold climatic conditions (Bramlage et al., [1978](#); Staniak et al., [2021](#); Szczerba et al., [2021](#); Tyagi & Tripathi, [1983](#)), although cold tolerance varies across maturity groups and among cultivars (Alsajri et al., [2019](#); Wang et al., [2023](#)). 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 cold winters (Matsushita et al., [2020](#); Owen, [2005](#)), and any soybean volunteers can be effectively controlled by mechanical methods or appropriate chemical control (Bond & Walker, [2009](#); Jhala et al., [2013](#); Soltani et al., [2019](#)). Owing to this, soybean plants are often not considered a problematic volunteer in temperate climates (Jhala et al., [2021](#)). 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 the two-event stack soybean and the observed reduction in crop development (see Section [3.4.2.5](#)) will provide a selective advantage to soybean plants, except when they are exposed to HPPD inhibitor-, glufosinate-, glyphosate- and/or 2,4-D-containing herbicides or infested by nematode pests that are susceptible to the Cry14Ab-1 protein. However, if this were to occur this fitness advantage would 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 will not overall affect the persistence and invasiveness of the GM plant.

³³Dossier: Part II – Section 5; additional information: 13/3/2025, 22/7/2025.

The GMO Panel concludes that it is very unlikely that the two-event stack soybean will differ from conventional soybean hybrid varieties in their 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 two-event stack soybean seeds.

3.4.4.2 | *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.

3.4.4.2.1 | *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 (see 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 concerns 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.

The applicant submitted an updated bioinformatic analysis for each of the single events to assess the possibility for HGT by homologous recombination. This information confirms the assessments provided in the context of previous Scientific Opinions of the single events (EFSA GMO Panel, 2017a (AP106-DAS-44406), 2021 (AP153-GMB151)).

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.

3.4.4.2.2 | *Plant-to-plant gene transfer*

The potential for occasional feral two-event soybean GMB151 × 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 GMB151 × 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* (Zhang et al., 2023). Although wild relatives exist elsewhere, no wild relatives of the subgenus *Soja* have been reported in Europe so far (Dorokhov et al., 2004; Lu, 2005). Therefore, potential 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% (Abud et al., 2007; Lu, 2005; OECD, 2000; Ray et al., 2003; Yoshimura et al., 2006), 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 (Ahrent & Caviness, 1994; Caviness, 1966; Gumisiriza & Rubaihayo, 1978; Kikuchi et al., 1993; Lu, 2005; Ray et al., 2003).

The potential of spilled soybean seeds to establish, grow and produce pollen is extremely low and transient (see Section 3.4.4.1). 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 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 3.4.4.1.

3.4.4.3 | *Interactions of the GM plant with target organisms*

Taking the scope of this application (no cultivation) potential interactions of occasional feral two-event stack soybean plants arising from seed import spills with the target organisms are not considered a relevant issue.

3.4.4.4 | *Interactions of the GM plant with non-target organisms*

The environmental risk assessment considers potential effects of the GM plant on populations of non-target organisms, defined as all those species directly or indirectly exposed to the GM plant and which are not targets of the newly expressed metabolites it expresses. The GMO Panel evaluated the potential hazards of the NEPs and considered that the environmental exposure of non-target organisms to spilled GM soybean material or occasional feral GM soybean plants arising from spilled soybean GMB151 × DAS-44406-6 seeds will be limited. Additionally, ingested proteins are typically degraded before

entering the environment through manure and faeces of animals fed with GM soybean (Harmon & Swanson, 2020; Miner-Williams et al., 2014; Mok & Urschel, 2020; Santos-Hernández et al., 2018; van Bruchem et al., 1985), and the data provided for the assessment of protein stability in the context of the assessment of the single events (see Section 3.4.3.1) supports that also the NEPs will be degraded. As compared to non-GM modified soybean, the GMO Panel considers that potential interactions of soybean GMB151 × DAS-44406-6 with non-target organisms do not raise any safety environmental concern.

3.4.4.5 | Interactions with biogeochemical cycles

Biogeochemical cycles encompass the microbiologically mediated movement, transformation and storage of carbon, nitrogen and other compounds that are considered here for the receiving environments. The GMO Panel evaluated the potential hazards of the NEPs and considered that the environmental exposure to spilled GM soybean material or occasional feral GM soybean plants arising from spilled soybean GMB151 × DAS-44406-6 seeds will be limited, whereas exposure to manure and faeces of animals fed with soybean GMB151 × DAS-44406-6 material is expected to be higher. However, ingested proteins are typically degraded before entering the environment through manure and faeces of animals fed with GM soybean (Harmon & Swanson, 2020; Miner-Williams et al., 2014; Mok & Urschel, 2020; Santos-Hernández et al., 2018; van Bruchem et al., 1985), and the data provided for the assessment of protein stability in the context of the assessment of the single events (see Section 3.4.3.1) supports that also the NEPs will be degraded. As compared to non-GM soybean, the GMO Panel considers that potential interactions of soybean GMB151 × DAS-44406-6 with biogeochemical cycles do not raise any environmental safety concern.

3.4.4.6 | Conclusion of the environmental risk assessment

The GMO Panel concludes that it is unlikely that the two-event stack soybean GMB151 × DAS-44406-6 would differ from conventional soybean varieties in its ability to persist under European environmental conditions. Taking into the account the scope of application GMFF-2024-21774, interactions of occasional feral two-event stack soybean plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from the two-event stack soybean to bacteria does not indicate a safety concern. Therefore, considering the introduced traits, the outcome of the agronomic and phenotypic analysis, and the routes and levels of exposure, the GMO Panel concludes that the two-event stack soybean would not raise safety concerns in the event of release of processed GM soybean GMB151 × DAS-44406-6 or the accidental spillage of viable GM soybean seeds, into the environment.

3.5 | Post-market monitoring

3.5.1 | Post-market monitoring of GM food/feed

The GMO Panel concluded that the two-event stack soybean GMB151 × DAS-44406-6, as described in this application, does not raise any nutritional concern and is as safe as the comparator and the non-GM reference varieties tested (Section 3.4.3). Therefore, the GMO Panel considers that post-market monitoring of food and feed from the two-event stack soybean, as described in this application, is not necessary.

3.5.2 | Post-market environmental monitoring

The objectives of a post-market environmental monitoring (PMEM) plan, according to Annex VII of Directive 2001/18/EC, are: (i) 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 (ii) 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 two-event stack soybean GMB151 × DAS-44406-6, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for the two-event stack soybean GMB151 × 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 co-ordinating system established by CropLife Europe 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., 2008). The applicant proposes to submit a PMEM report on an annual basis for the duration 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 the two-event stack soybean GMB151 × DAS-44406-6. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

3.5.3 | Conclusions on post-market monitoring

No post-market monitoring of food and feed is necessary. The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of the two-event stack soybean.

4 | OVERALL CONCLUSIONS

The GMO Panel was asked to carry out a scientific assessment of soybean GMB151 × DAS-44406-6 for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003.

No new information was identified on the two single soybean events (GMB151 and DAS-44406-6) that would lead to a modification of the original conclusions on their safety.

The molecular characterisation, the 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/feed safety and nutritional concerns. The GMO Panel concludes that the two-event stack soybean, as described in this application, does not raise any nutritional concern and is as safe as its comparator and the selected non-GM reference varieties.

The GMO Panel concludes that additional environmental effects as compared to conventional soybean resulting from the release of the two-event stack soybean into the environment are unlikely.

The literature searches did not identify any relevant publications on soybean GMB151 × DAS-44406-6. Therefore, the GMO Panel does not identify any safety issue pertaining to the intended uses of the two-event stack soybean.

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. Given the absence of safety and nutritional concerns for foods and feeds from the two-event stack soybean, the GMO Panel considers that PMM of these products is not necessary. The PMEM plan and reporting intervals are in line with the intended uses of the two-event stack soybean. In conclusion, the GMO Panel considers that soybean GMB151 × DAS-44406-6, as described in this application, is as safe as the non-GM comparator and the selected non-GM reference varieties with respect to potential effects on human and animal health and the environment.

ABBREVIATIONS

2,4-D	2,4-dichlorophenoxyacetic acid
AAD-12	aryloxyalkanoate dioxygenase-12
ADF	acid detergent fibre
DCP	2,4-dichlorophenol
DDE	daily dietary intake
ELISA	enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphat synthase
ERA	environmental risk assessment
GM	genetically modified
GMO	genetically modified organism
GMO Panel	EFSA Panel on Genetically Modified Organisms
HGT	horizontal gene transfer
HR	homologous recombination
IFT	isoxaflutole
LOD	limit of detection
LOQ	limit of quantification
MS	Member States
NDF	neutral detergent fibre
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PAT	phosphinothricin-acetyl-transferase
PCR	polymerase chain reaction
PMEM	post-market environmental monitoring
SCN	Soybean Cyst Nematode

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REQUESTOR

Competent Authority of Belgium

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX A

Additional studies

List of additional studies performed by or on behalf of the applicant with regard to the evaluation of the safety of soybean GMB151 × DAS44406 for humans, animals or the environment.

Study identification	Title
22-RSSB0703	GMB151 × DAS44406 Soybean – Seed Germination Potential, 2022

APPENDIX B

Protein expression data

TABLE B.1 Mean, standard deviation and range of protein levels (µg/g dry weight) from soybean GMB151 × DAS44406 (treated) and GMB151 and DAS44406 (treated), from field trials performed across four locations in the United States during the 2022 growing season ($n = 12$).^a

Protein	Event(s)	Leaf (BBCH 13–14)	Leaf (BBCH 16–17)	Flower (BBCH 60–66)	Leaf (BBCH 60–66)	Root (BBCH 60–66)	Forage (BBCH 71–79)	Leaf (BBCH 71–79)	Root (BBCH 71–79)	Whole plant (BBCH 81–89) ^{a,b}	Seeds (BBCH 89–99)
Cry14Ab-1	GMB151 × DAS44406	65.55 ^c ± 52.42 ^d (3.54–181.26) ^e	59.56 ± 52.56 (17.82–202.71)	41.70 ± 18.22 (14.14–61.75)	106.12 ± 44.80 (61.48–183.96)	14.90 ± 19.86 (0.82–68.60)	58.91 ± 38.50 (17.39–155.78)	86.45 ± 53.02 (10.27–156.55)	39.60 ± 35.28 (0.79–100.71)	54.73 ± 16.61 (29.60–88.33)	103.70 ± 14.49 (80.35–128.94)
	GMB151	44.33 ± 33.69 (8.71–116.96)	69.17 ± 48.15 (4.67–153.73)	32.21 ± 19.71 (9.13–66.90)	70.60 ± 46.83 (32.06–191.29)	22.08 ± 23.71 (0.59–80.09)	65.03 ± 30.03 (29.76–145.06)	126.11 ± 55.85 (50.10–227.37)	80.86 ± 128.12 (0.31–447.17)	47.63 ± 19.33 (26.71–88.07)	94.6 ± 14.44 (65.91–118.00)
HPPD-4	GMB151 × DAS44406	158.79 ± 50.55 (83.58–229.15)	140.18 ± 55.08 (61.11–252.53)	29.48 ± 4.87 (21.78–39.91)	123.92 ± 65.14 (27.78–210.02)	8.14 ± 2.68 (5.00–12.16)	39.83 ± 18.76 (18.71–83.81)	68.99 ± 42.37 (27.35–188.02)	< 11.51 ± 7.6 (< 1.09–27.03) ^f	16.31 ± 6.90 (8.69–29.24)	12.74 ± 1.93 (10.33–16.50)
	GMB151	128.95 ± 72.20 (42.64–286.68)	161.26 ± 49.45 (83.50–247.46)	30.63 ± 4.44 (20.49–35.64)	108.15 ± 53.22 (15.18–189.11)	7.68 ± 1.17 (5.75–9.59)	39.39 ± 16.21 (15.47–69.43)	56.21 ± 18.22 (29.24–84.75)	9.92 ± 7.69 (2.25–28.47)	16.18 ± 8.95 (6.30–33.53)	10.60 ± 2.43 (7.37–14.41)
PAT	GMB151 × DAS44406	11.36 ± 3.56 (6.20–18.58)	11.19 ± 2.90 (5.82–15.22)	6.96 ± 0.98 (4.57–8.45)	15.32 ± 2.08 (10.74–18.48)	2.32 ± 0.75 (1.19–3.38)	6.03 ± 1.59 (3.91–8.48)	10.31 ± 2.03 (7.02–13.64)	2.41 ± 0.75 (1.27–3.85)	2.49 ± 0.47 (1.80–3.47)	2.82 ± 0.40 (2.04–3.32)
	DAS44406	12.61 ± 2.60 (6.87–16.81)	11.32 ± 3.31 (6.52–16.99)	7.67 ± 0.59 (6.16–8.30)	16.34 ± 2.76 (9.85–20.75)	2.60 ± 0.60 (1.61–3.32)	6.56 ± 1.47 (4.38–8.40)	11.77 ± 1.78 (9.17–14.76)	2.50 ± 0.83 (1.60–4.64)	2.54 ± 0.55 (1.83–3.80)	3.06 ± 0.33 (2.26–3.46)
AAD-12	GMB151 × DAS44406	122.51 ± 33.44 (71.46–172.36)	128.52 ± 30.88 (85.12–190.08)	66.13 ± 11.78 (41.57–82.34)	94.18 ± 35.05 (41.05–157.29)	39.01 ± 15.36 (14.91–60.33)	69.22 ± 16.50 (40.74–99.79)	113.24 ± 23.07 (80.70–146.99)	43.93 ± 20.41 (18.49–83.55)	44.20 ± 8.06 (30.59–59.21)	66.68 ± 11.31 (46.23–80.89)
	DAS44406	160.20 ± 29.20 (103.55–207.23)	131.12 ± 57.68 (45.40–212.93)	62.28 ± 8.24 (46.12–74.83)	88.34 ± 36.63 (37.18–137.62)	39.28 ± 14.98 (17.15–61.27)	70.72 ± 18.93 (45.79–95.62)	108.23 ± 35.22 (28.59–153.05)	39.92 ± 18.89 (20.17–86.43)	41.73 ± 12.87 (26.12–70.26)	68.09 ± 9.35 (54.84–82.82)
2mEPSPS	GMB151 × DAS44406	36.82 ± 26.40 (18.39–115.46)	100.73 ± 54.62 (35.74–214.07)	128.50 ± 11.75 (99.94–144.50)	61.89 ± 21.14 (35.46–96.14)	11.01 ± 8.89 (3.65–29.93)	71.00 ± 44.85 (12.55–130.38)	115.01 ± 64.87 (39.62–246.05)	13.04 ± 12.06 (2.70–38.70)	< 13.45 ± 12.14 (< 1.31–32.32) ^g	< 3.58 ± 0.08 (< 3.52–3.71) ^f
	DAS44406	49.05 ± 19.11 (22.32–79.76)	113.40 ± 65.23 (40.41–251.18)	140.68 ± 23.69 (89.82–166.39)	85.54 ± 36.24 (44.18–162.74)	10.22 ± 11.44 (3.13–43.90)	82.16 ± 54.61 (6.77–151.86)	104.57 ± 56.78 (51.29–199.71)	< 7.23 ± 4.72 (< 1.28–18.39) ^g	< 11.91 ± 13.69 (< 1.34–47.15) ^g	< 3.58 ± 0.17 (< 3.42–4.08) ^f

^aNumber of samples is $n = 12$ except in: Whole Plant (R6) tissue for the event GMB151 × DAS44406 where $n = 16$.^bWhole plant (R6) samples of events GMB151 and DAS4440 from site 03 were excluded from the mean calculation since they were not of the expected identity.^cMean.^dStandard deviation.^eRange.^fSome, but not all, sample results were below the lower limit of quantification (LLOQ). A value equal to the LLOQ value was used for those samples to calculate the mean and standard deviation. The LLOQ value of the method is expressed in mg/ml. To calculate the mean expression level, this value was converted to mg/g dry weight (DW). This calculation took into account the exact weight of the sample. As each sample was individually weighed, the slight variation in weight also resulted in slight differences in LLOQ values expressed in mg/g DW. When the '<' symbol is used the value reported is the value of the LLOQ for that specific tissue.^gSome, but not all, sample results were below the LOD. A value equal to the LOD value was used for those samples to calculate the mean and standard deviation. The LOD value of the method is expressed in mg/ml. To calculate the mean expression level, this value was converted to mg/g dry weight (DW). This calculation took into account the exact weight of the sample. As each sample was individually weighed, the slight variation in weight also resulted in slight differences in LOD values expressed in mg/g DW. When the '<' symbol is used the value reported is the value of the LOD for that specific tissue.

APPENDIX C

Animal dietary exposure

TABLE C.1 Dietary exposure to Cry14Ab-1 protein in farm and companion animals, based on the consumption of soybean full fat seed.

Animal species	Animal body weight	Total daily intake of FEED	Inclusion rate of SOYBEAN FULL FAT SEED	Daily dietary intake of SOYBEAN FULL FAT SEED	NEP level Cry14Ab-1	Daily dietary exposure to NEP	
Category	kg	kg ^{DM} /animal	IR %	g ^{DM} /kg ^{BW}	μg ^{NEP} /g ^{DM}	μg ^{NEP} /kg ^{BW}	mg ^{NEP} /kg ^{BW}
Dairy cow	650	25	10	3.85	103.70	398.85	0.40
Beef cattle	500	12	10	2.40	103.70	248.88	0.25
Dairy Sheep	80	2.8	14	4.90	103.70	508.13	0.51
Dairy Goat	60	3.4	11	6.23	103.70	646.40	0.65
Rabbit	2	0.15	20	15.00	103.70	1555.50	1.56
Fattening pig	100	3	20	6.00	103.70	622.20	0.62
Lactating sow	200	6	10	3.00	103.70	311.10	0.31
Piglet	20	1	30	15.00	103.70	1555.50	1.56
Broiler	2	0.158	20	15.80	103.70	1638.46	1.64
Laying hens	1.9	0.13	15	10.26	103.70	1064.29	1.06
Turkey	7	0.5	15	10.71	103.70	1111.07	1.11
Salmon	5	0.03	NA	NA	NA	NA	NA
Carp	1	0.02	NA	NA	NA	NA	NA
Cat	4	0.06	NA	NA	NA	NA	NA
Dog	25	0.36	30	4.32	103.70	447.98	0.45
Horse	450	9	NA	NA	NA	NA	NA

TABLE C.2 Dietary exposure to HPPD-4 protein in farm and companion animals, based on the consumption of soybean full fat seed.

Animal species	Animal body weight	Total daily intake of FEED	Inclusion rate of SOYBEAN FULL FAT SEED	Daily dietary intake of SOYBEAN FULL FAT SEED	NEP level HPPD-4	Daily dietary exposure to NEP	
Category	kg	kg ^{DM} /animal	IR %	g ^{DM} /kg ^{BW}	μg ^{NEP} /g ^{DM}	μg ^{NEP} /kg ^{BW}	mg ^{NEP} /kg ^{BW}
Dairy cow	650	25	10	3.85	12.74	49.00	0.05
Beef cattle	500	12	10	2.40	12.74	30.58	0.03
Dairy Sheep	80	2.8	14	4.90	12.74	62.43	0.06
Dairy Goat	60	3.4	11	6.23	12.74	79.41	0.08
Rabbit	2	0.15	20	15.00	12.74	191.10	0.19
Fattening pig	100	3	20	6.00	12.74	76.44	0.08
Lactating sow	200	6	10	3.00	12.74	38.22	0.04
Piglet	20	1	30	15.00	12.74	191.10	0.19
Broiler	2	0.158	20	15.80	12.74	201.29	0.20
Laying hens	1.9	0.13	15	10.26	12.74	130.75	0.13
Turkey	7	0.5	15	10.71	12.74	136.50	0.14
Salmon	5	0.03	NA	NA	NA	NA	NA
Carp	1	0.02	NA	NA	NA	NA	NA
Cat	4	0.06	NA	NA	NA	NA	NA
Dog	25	0.36	30	4.32	12.74	55.04	0.06
Horse	450	9	NA	NA	NA	NA	NA

TABLE C.3 Dietary exposure to PAT protein in farm and companion animals, based on the consumption of soybean full fat seed.

Animal species	Animal body weight	Total daily intake of FEED	Inclusion rate of SOYBEAN FULL FAT SEED	Daily dietary intake of SOYBEAN FULL FAT SEED	NEP level PAT	Daily dietary exposure to NEP	
Category	kg	kg ^{DM} /animal	IR %	g ^{DM} /kg ^{BW}	μg ^{NEP} /g ^{DM}	μg ^{NEP} /kg ^{BW}	mg ^{NEP} /kg ^{BW}
Dairy cow	650	25	10	3.85	2.82	10.85	0.01
Beef cattle	500	12	10	2.40	2.82	6.77	0.01
Dairy Sheep	80	2.8	14	4.90	2.82	13.82	0.01
Dairy Goat	60	3.4	11	6.23	2.82	17.58	0.02
Rabbit	2	0.15	20	15.00	2.82	42.30	0.04
Fattening pig	100	3	20	6.00	2.82	16.92	0.02
Lactating sow	200	6	10	3.00	2.82	8.46	0.01
Piglet	20	1	30	15.00	2.82	42.30	0.04
Broiler	2	0.158	20	15.80	2.82	44.56	0.04
Laying hens	1.9	0.13	15	10.26	2.82	28.94	0.03
Turkey	7	0.5	15	10.71	2.82	30.21	0.03
Salmon	5	0.03	NA	NA	NA	NA	NA
Carp	1	0.02	NA	NA	NA	NA	NA
Cat	4	0.06	NA	NA	NA	NA	NA
Dog	25	0.36	30	4.32	2.82	12.18	0.01
Horse	450	9	NA	NA	NA	NA	NA

TABLE C.4 Dietary exposure to AAD-12 protein in farm and companion animals, based on the consumption of soybean full fat seed.

Animal species	Animal body weight	Total daily intake of FEED	Inclusion rate of SOYBEAN FULL FAT SEED	Daily dietary intake of SOYBEAN FULL FAT SEED	NEP level AAD-12	Daily dietary exposure to NEP	
Category	kg	kg ^{DM} /animal	IR %	g ^{DM} /kg ^{BW}	μg ^{NEP} /g ^{DM}	μg ^{NEP} /kg ^{BW}	mg ^{NEP} /kg ^{BW}
Dairy cow	650	25	10	3.85	66.68	256.46	0.26
Beef cattle	500	12	10	2.40	66.68	160.03	0.16
Dairy Sheep	80	2.8	14	4.90	66.68	326.73	0.33
Dairy Goat	60	3.4	11	6.23	66.68	415.64	0.42
Rabbit	2	0.15	20	15.00	66.68	1000.20	1.00
Fattening pig	100	3	20	6.00	66.68	400.08	0.40
Lactating sow	200	6	10	3.00	66.68	200.04	0.20
Piglet	20	1	30	15.00	66.68	1000.20	1.00
Broiler	2	0.158	20	15.80	66.68	1053.54	1.05
Laying hens	1.9	0.13	15	10.26	66.68	684.35	0.68
Turkey	7	0.5	15	10.71	66.68	714.43	0.71
Salmon	5	0.03	NA	NA	NA	NA	NA
Carp	1	0.02	NA	NA	NA	NA	NA
Cat	4	0.06	NA	NA	NA	NA	NA
Dog	25	0.36	30	4.32	66.68	288.06	0.29
Horse	450	9	NA	NA	NA	NA	NA

TABLE C.5 Dietary exposure to 2mEPSPS protein in farm and companion animals, based on the consumption of soybean full fat seed.

Animal species	Animal body weight	Total daily intake of FEED	Inclusion rate of SOYBEAN FULL FAT SEED	Daily dietary intake of SOYBEAN FULL FAT SEED	NEP level 2mEPSPS	Daily dietary exposure to NEP	
Category	kg	kg ^{DM} /animal	IR %	g ^{DM} /kg ^{BW}	μg ^{NEP} /g ^{DM}	μg ^{NEP} /kg ^{BW}	mg ^{NEP} /kg ^{BW}
Dairy cow	650	25	10	3.85	3.58	13.77	0.01
Beef cattle	500	12	10	2.40	3.58	8.59	0.01
Dairy Sheep	80	2.8	14	4.90	3.58	17.54	0.02
Dairy Goat	60	3.4	11	6.23	3.58	22.32	0.02
Rabbit	2	0.15	20	15.00	3.58	53.70	0.05
Fattening pig	100	3	20	6.00	3.58	21.48	0.02
Lactating sow	200	6	10	3.00	3.58	10.74	0.01
Piglet	20	1	30	15.00	3.58	53.70	0.05
Broiler	2	0.158	20	15.80	3.58	56.56	0.06
Laying hens	1.9	0.13	15	10.26	3.58	36.74	0.04
Turkey	7	0.5	15	10.71	3.58	38.36	0.04
Salmon	5	0.03	NA	NA	NA	NA	NA
Carp	1	0.02	NA	NA	NA	NA	NA
Cat	4	0.06	NA	NA	NA	NA	NA
Dog	25	0.36	30	4.32	3.58	15.47	0.02
Horse	450	9	NA	NA	NA	NA	NA

TABLE C.6 Dietary exposure to Cry14Ab-1protein in farm and companion animals, based on the consumption of soybean meal.

Animal species	Animal body weight	Total daily intake of FEED	Inclusion rate of SOYBEAN MEAL	Daily dietary intake of SOYBEAN MEAL	NEP level Cry14Ab-1	Daily dietary exposure to NEP	
Category	kg	kg ^{DM} /animal	IR %	g ^{DM} /kg ^{BW}	μg ^{NEP} /g ^{DM}	μg ^{NEP} /kg ^{BW}	mg ^{NEP} /kg ^{BW}
Dairy cow	650	25	10	3.85	136.88	1316.15	1.32
Beef cattle	500	12	10	2.40	136.88	657.02	0.66
Dairy Sheep	80	2.8	14	4.90	136.88	958.16	0.96
Dairy Goat	60	3.4	11	6.23	136.88	1939.13	1.94
Rabbit	2	0.15	20	15.00	136.88	2463.84	2.46
Fattening pig	100	3	20	6.00	136.88	1231.92	1.23
Lactating sow	200	6	10	3.00	136.88	1231.92	1.23
Piglet	20	1	30	15.00	136.88	1505.68	1.51
Broiler	2	0.158	20	15.80	136.88	4325.41	4.33
Laying hens	1.9	0.13	15	10.26	136.88	2341.37	2.34
Turkey	7	0.5	15	10.71	136.88	4399.71	4.40
Salmon	5	0.03	NA	NA	NA	NA	NA
Carp	1	0.02	NA	NA	136.88	1149.79	1.15
Cat	4	0.06	NA	NA	136.88	605.69	0.61
Dog	25	0.36	30	4.32	136.88	591.32	0.59
Horse	450	9	NA	NA	NA	NA	NA

TABLE C.7 Dietary exposure to HPPD-4 protein in farm and companion animals, based on the consumption of soybean meal.

Animal species	Animal body weight	Total daily intake of FEED	Inclusion rate of SOYBEAN MEAL	Daily dietary intake of SOYBEAN MEAL	NEP level HPPD-4	Daily dietary exposure to NEP	
Category	kg	kg ^{DM} /animal	IR %	g ^{DM} /kg ^{BW}	μg ^{NEP} /g ^{DM}	μg ^{NEP} /kg ^{BW}	mg ^{NEP} /kg ^{BW}
Dairy cow	650	25	10	3.85	16.82	161.73	0.16
Beef cattle	500	12	10	2.40	16.82	80.74	0.08
Dairy Sheep	80	2.8	14	4.90	16.82	117.74	0.12
Dairy Goat	60	3.4	11	6.23	16.82	238.28	0.24
Rabbit	2	0.15	20	15.00	16.82	302.76	0.30
Fattening pig	100	3	20	6.00	16.82	151.38	0.15
Lactating sow	200	6	10	3.00	16.82	151.38	0.15
Piglet	20	1	30	15.00	16.82	185.02	0.19
Broiler	2	0.158	20	15.80	16.82	531.51	0.53
Laying hens	1.9	0.13	15	10.26	16.82	287.71	0.29
Turkey	7	0.5	15	10.71	16.82	540.64	0.54
Salmon	5	0.03	NA	NA	NA	NA	NA
Carp	1	0.02	NA	NA	16.82	141.29	0.14
Cat	4	0.06	NA	NA	16.82	74.43	0.07
Dog	25	0.36	30	4.32	16.82	72.66	0.07
Horse	450	9	NA	NA	NA	NA	NA

TABLE C.8 Dietary exposure to PAT protein in farm and companion animals, based on the consumption of soybean meal.

Animal species	Animal body weight	Total daily intake of FEED	Inclusion rate of SOYBEAN MEAL	Daily dietary intake of SOYBEAN MEAL	NEP level PAT	Daily dietary exposure to NEP	
Category	kg	kg ^{DM} /animal	IR %	g ^{DM} /kg ^{BW}	μg ^{NEP} /g ^{DM}	μg ^{NEP} /kg ^{BW}	mg ^{NEP} /kg ^{BW}
Dairy cow	650	25	10	3.85	3.72	35.77	0.04
Beef cattle	500	12	10	2.40	3.72	17.86	0.02
Dairy Sheep	80	2.8	14	4.90	3.72	26.04	0.03
Dairy Goat	60	3.4	11	6.23	3.72	52.70	0.05
Rabbit	2	0.15	20	15.00	3.72	66.96	0.07
Fattening pig	100	3	20	6.00	3.72	33.48	0.03
Lactating sow	200	6	10	3.00	3.72	33.48	0.03
Piglet	20	1	30	15.00	3.72	40.92	0.04
Broiler	2	0.158	20	15.80	3.72	117.55	0.12
Laying hens	1.9	0.13	15	10.26	3.72	63.63	0.06
Turkey	7	0.5	15	10.71	3.72	119.57	0.12
Salmon	5	0.03	NA	NA	NA	NA	NA
Carp	1	0.02	NA	NA	3.72	31.25	0.03
Cat	4	0.06	NA	NA	3.72	16.46	0.02
Dog	25	0.36	30	4.32	3.72	16.07	0.02
Horse	450	9	NA	NA	NA	NA	NA

TABLE C.9 Dietary exposure to AAD-12 protein in farm and companion animals, based on the consumption of soybean meal.

Animal species	Animal body weight	Total daily intake of FEED	Inclusion rate of SOYBEAN MEAL	Daily dietary intake of SOYBEAN MEAL	NEP level AAD-12	Daily dietary exposure to NEP	
Category	kg	kg ^{DM} /animal	IR %	g ^{DM} /kg ^{BW}	μg ^{NEP} /g ^{DM}	μg ^{NEP} /kg ^{BW}	mg ^{NEP} /kg ^{BW}
Dairy cow	650	25	10	3.85	88.02	846.35	0.85
Beef cattle	500	12	10	2.40	88.02	422.50	0.42
Dairy Sheep	80	2.8	14	4.90	88.02	616.14	0.62
Dairy Goat	60	3.4	11	6.23	88.02	1246.95	1.25
Rabbit	2	0.15	20	15.00	88.02	1584.36	1.58
Fattening pig	100	3	20	6.00	88.02	792.18	0.79
Lactating sow	200	6	10	3.00	88.02	792.18	0.79
Piglet	20	1	30	15.00	88.02	968.22	0.97
Broiler	2	0.158	20	15.80	88.02	2781.43	2.78
Laying hens	1.9	0.13	15	10.26	88.02	1505.61	1.51
Turkey	7	0.5	15	10.71	88.02	2829.21	2.83
Salmon	5	0.03	NA	NA	NA	NA	NA
Carp	1	0.02	NA	NA	88.02	739.37	0.74
Cat	4	0.06	NA	NA	88.02	389.49	0.39
Dog	25	0.36	30	4.32	88.02	380.25	0.38
Horse	450	9	NA	NA	NA	NA	NA

TABLE C.10 Dietary exposure to 2mEPSPS protein in farm and companion animals, based on the consumption of soybean meal.

Animal species	Animal body weight	Total daily intake of FEED	Inclusion rate of SOYBEAN MEAL	Daily dietary intake of SOYBEAN MEAL	NEP level 2mEPSPS	Daily dietary exposure to NEP	
Category	kg	kg ^{DM} /animal	IR %	g ^{DM} /kg ^{BW}	μg ^{NEP} /g ^{DM}	μg ^{NEP} /kg ^{BW}	mg ^{NEP} /kg ^{BW}
Dairy cow	650	25	10	3.85	4.73	45.48	0.05
Beef cattle	500	12	10	2.40	4.73	22.70	0.02
Dairy Sheep	80	2.8	14	4.90	4.73	33.11	0.03
Dairy Goat	60	3.4	11	6.23	4.73	67.01	0.07
Rabbit	2	0.15	20	15.00	4.73	85.14	0.09
Fattening pig	100	3	20	6.00	4.73	42.57	0.04
Lactating sow	200	6	10	3.00	4.73	42.57	0.04
Piglet	20	1	30	15.00	4.73	52.03	0.05
Broiler	2	0.158	20	15.80	4.73	149.47	0.15
Laying hens	1.9	0.13	15	10.26	4.73	80.91	0.08
Turkey	7	0.5	15	10.71	4.73	152.04	0.15
Salmon	5	0.03	NA	NA	NA	NA	NA
Carp	1	0.02	NA	NA	4.73	39.73	0.04
Cat	4	0.06	NA	NA	4.73	20.93	0.02
Dog	25	0.36	30	4.32	4.73	20.43	0.02
Horse	450	9	NA	NA	NA	NA	NA

TABLE C.11 Dietary exposure to Cry14Ab-1 protein in farm and companion animals, based on the consumption of soybean hull.

Animal species	Animal body weight	Total daily intake of FEED	Inclusion rate of SOYBEAN hull	Daily dietary intake of SOYBEAN hull	NEP level Cry14Ab-1	Daily dietary exposure to NEP	
Category	kg	kg ^{DM} /animal	IR %	g ^{DM} /kg ^{BW}	μg ^{NEP} /g ^{DM}	μg ^{NEP} /kg ^{BW}	mg ^{NEP} /kg ^{BW}
Dairy cow	650	25	10	3.85	35.26	135.62	0.14
Beef cattle	500	12	10	2.40	35.26	84.62	0.08
Dairy Sheep	80	2.8	14	4.90	35.26	555.35	0.56
Dairy Goat	60	3.4	11	6.23	35.26	1218.82	1.22
Rabbit	2	0.15	20	15.00	35.26	859.46	0.86
Fattening pig	100	3	20	6.00	35.26	105.78	0.11
Lactating sow	200	6	10	3.00	35.26	105.78	0.11
Piglet	20	1	3	1.50	35.26	52.89	0.05
Broiler	2	0.158	20	15.80	35.26	278.55	0.28
Laying hens	1.9	0.13	15	10.26	35.26	120.63	0.12
Turkey	7	0.5	15	10.71	NA	NA	NA
Salmon	5	0.03	NA	NA	NA	NA	NA
Carp	1	0.02	NA	NA	NA	NA	NA
Cat	4	0.06	NA	NA	35.26	74.05	0.07
Dog	25	0.36	30	4.32	35.26	38.08	0.04
Horse	450	9	NA	NA	35.26	528.90	0.53

TABLE C.12 Dietary exposure to HPPD-4 protein in farm and companion animals, based on the consumption of soybean hull.

Animal species	Animal body weight	Total daily intake of FEED	Inclusion rate of SOYBEAN hull	Daily dietary intake of SOYBEAN hull	NEP level HPPD-4	Daily dietary exposure to NEP	
Category	kg	kg ^{DM} /animal	IR %	g ^{DM} /kg ^{BW}	μg ^{NEP} /g ^{DM}	μg ^{NEP} /kg ^{BW}	mg ^{NEP} /kg ^{BW}
Dairy cow	650	25	10	3.85	4.33	16.65	0.02
Beef cattle	500	12	10	2.40	4.33	10.39	0.01
Dairy Sheep	80	2.8	14	4.90	4.33	68.20	0.07
Dairy Goat	60	3.4	11	6.23	4.33	149.67	0.15
Rabbit	2	0.15	20	15.00	4.33	105.54	0.11
Fattening pig	100	3	20	6.00	4.33	12.99	0.01
Lactating sow	200	6	10	3.00	4.33	12.99	0.01
Piglet	20	1	3	1.50	4.33	6.50	0.01
Broiler	2	0.158	20	15.80	4.33	34.21	0.03
Laying hens	1.9	0.13	15	10.26	4.33	14.81	0.01
Turkey	7	0.5	15	10.71	NA	NA	NA
Salmon	5	0.03	NA	NA	NA	NA	NA
Carp	1	0.02	NA	NA	NA	NA	NA
Cat	4	0.06	NA	NA	4.33	9.09	0.01
Dog	25	0.36	30	4.32	4.33	4.68	0.005
Horse	450	9	NA	NA	4.33	64.95	0.06

TABLE C.13 Dietary exposure to PAT protein in farm and companion animals, based on the consumption of soybean hull.

Animal species	Animal body weight	Total daily intake of FEED	Inclusion rate of SOYBEAN hull	Daily dietary intake of SOYBEAN hull	NEP level PAT	Daily dietary exposure to NEP	
Category	kg	kg ^{DM} /animal	IR %	g ^{DM} /kg ^{BW}	μg ^{NEP} /g ^{DM}	μg ^{NEP} /kg ^{BW}	mg ^{NEP} /kg ^{BW}
Dairy cow	650	25	10	3.85	0.96	3.69	0.004
Beef cattle	500	12	10	2.40	0.96	2.30	0.002
Dairy Sheep	80	2.8	14	4.90	0.96	15.12	0.015
Dairy Goat	60	3.4	11	6.23	0.96	33.18	0.033
Rabbit	2	0.15	20	15.00	0.96	23.40	0.023
Fattening pig	100	3	20	6.00	0.96	2.88	0.003
Lactating sow	200	6	10	3.00	0.96	2.88	0.003
Piglet	20	1	3	1.50	0.96	1.44	0.001
Broiler	2	0.158	20	15.80	0.96	7.58	0.008
Laying hens	1.9	0.13	15	10.26	0.96	3.28	0.003
Turkey	7	0.5	15	10.71	NA	NA	NA
Salmon	5	0.03	NA	NA	NA	NA	NA
Carp	1	0.02	NA	NA	NA	NA	NA
Cat	4	0.06	NA	NA	0.96	2.02	0.002
Dog	25	0.36	30	4.32	0.96	1.04	0.001
Horse	450	9	NA	NA	0.96	14.40	0.014

TABLE C.14 Dietary exposure to AAD-12 protein in farm and companion animals, based on the consumption of soybean hull.

Animal species	Animal body weight	Total daily intake of FEED	Inclusion rate of SOYBEAN hull	Daily dietary intake of SOYBEAN hull	NEP level AAD-12	Daily dietary exposure to NEP	
Category	kg	kg ^{DM} /animal	IR %	g ^{DM} /kg ^{BW}	μg ^{NEP} /g ^{DM}	μg ^{NEP} /kg ^{BW}	mg ^{NEP} /kg ^{BW}
Dairy cow	650	25	10	3.85	22.67	87.19	0.09
Beef cattle	500	12	10	2.40	22.67	54.41	0.05
Dairy Sheep	80	2.8	14	4.90	22.67	357.05	0.36
Dairy Goat	60	3.4	11	6.23	22.67	783.63	0.78
Rabbit	2	0.15	20	15.00	22.67	552.58	0.55
Fattening pig	100	3	20	6.00	22.67	68.01	0.07
Lactating sow	200	6	10	3.00	22.67	68.01	0.07
Piglet	20	1	3	1.50	22.67	34.01	0.03
Broiler	2	0.158	20	15.80	22.67	179.09	0.18
Laying hens	1.9	0.13	15	10.26	22.67	77.56	0.08
Turkey	7	0.5	15	10.71	NA	NA	NA
Salmon	5	0.03	NA	NA	NA	NA	NA
Carp	1	0.02	NA	NA	NA	NA	NA
Cat	4	0.06	NA	NA	22.67	47.61	0.05
Dog	25	0.36	30	4.32	22.67	24.48	0.02
Horse	450	9	NA	NA	22.67	340.05	0.34

TABLE C.15 Dietary exposure to 2mEPSPS protein in farm and companion animals, based on the consumption of soybean hull.

Animal species	Animal body weight	Total daily intake of FEED	Inclusion rate of SOYBEAN hull	Daily dietary intake of SOYBEAN hull	NEP level 2mEPSPS	Daily dietary exposure to NEP	
Category	kg	kg ^{DM} /animal	IR %	g ^{DM} /kg ^{BW}	μg ^{NEP} /g ^{DM}	μg ^{NEP} /kg ^{BW}	mg ^{NEP} /kg ^{BW}
Dairy cow	650	25	10	3.85	1.22	4.69	0.005
Beef cattle	500	12	10	2.40	1.22	2.93	0.003
Dairy Sheep	80	2.8	14	4.90	1.22	19.22	0.019
Dairy Goat	60	3.4	11	6.23	1.22	42.17	0.042
Rabbit	2	0.15	20	15.00	1.22	29.74	0.030
Fattening pig	100	3	20	6.00	1.22	3.66	0.004
Lactating sow	200	6	10	3.00	1.22	3.66	0.004
Piglet	20	1	3	1.50	1.22	1.83	0.002
Broiler	2	0.158	20	15.80	1.22	9.64	0.010
Laying hens	1.9	0.13	15	10.26	1.22	4.17	0.004
Turkey	7	0.5	15	10.71	NA	NA	NA
Salmon	5	0.03	NA	NA	NA	NA	NA
Carp	1	0.02	NA	NA	NA	NA	NA
Cat	4	0.06	NA	NA	1.22	2.56	0.003
Dog	25	0.36	30	4.32	1.22	1.32	0.001
Horse	450	9	NA	NA	1.22	18.30	0.018

TABLE C.16 Dietary exposure to Cry14Ab-1 protein in farm and companion animals, based on the consumption of soybean protein concentrate.

Animal species	Animal body weight	Total daily intake of FEED	Inclusion rate of SOYBEAN protein concentrate	Daily dietary intake of SOYBEAN protein concentrate	NEP level Cry14Ab-1	Daily dietary exposure to NEP	
Category	kg	kg ^{DM} /animal	IR %	g ^{DM} /kg ^{BW}	μg ^{NEP} /g ^{DM}	μg ^{NEP} /kg ^{BW}	mg ^{NEP} /kg ^{BW}
Dairy cow	650	25	10	3.85	NA	NA	NA
Beef cattle	500	12	10	2.40	NA	NA	NA
Dairy Sheep	80	2.8	14	4.90	NA	NA	NA
Dairy Goat	60	3.4	11	6.23	NA	NA	NA
Rabbit	2	0.15	20	15.00	NA	NA	NA
Fattening pig	100	3	20	6.00	NA	NA	NA
Lactating sow	200	6	10	3.00	NA	NA	NA
Piglet	20	1	30	15.00	214.66	965.97	0.97
Broiler	2	0.158	20	15.80	NA	NA	NA
Laying hens	1.9	0.13	15	10.26	NA	NA	NA
Turkey	7	0.5	15	10.71	NA	NA	NA
Salmon	5	0.03	NA	NA	214.66	128.80	0.13
Carp	1	0.02	NA	NA	NA	NA	NA
Cat	4	0.06	NA	NA	214.66	804.98	0.80
Dog	25	0.36	30	4.32	214.66	578.04	0.58
Horse	450	9	NA	NA	NA	NA	NA

TABLE C.17 Dietary exposure to HPPD-4 protein in farm and companion animals, based on the consumption of soybean protein concentrate.

Animal species	Animal body weight	Total daily intake of FEED	Inclusion rate of SOYBEAN protein concentrate	Daily dietary intake of SOYBEAN protein concentrate	NEP level HPPD-4	Daily dietary exposure to NEP	
Category	kg	kg ^{DM} /animal	IR %	g ^{DM} /kg ^{BW}	μg ^{NEP} /g ^{DM}	μg ^{NEP} /kg ^{BW}	mg ^{NEP} /kg ^{BW}
Dairy cow	650	25	10	3.85	NA	NA	NA
Beef cattle	500	12	10	2.40	NA	NA	NA
Dairy Sheep	80	2.8	14	4.90	NA	NA	NA
Dairy Goat	60	3.4	11	6.23	NA	NA	NA
Rabbit	2	0.15	20	15.00	NA	NA	NA
Fattening pig	100	3	20	6.00	NA	NA	NA
Lactating sow	200	6	10	3.00	NA	NA	NA
Piglet	20	1	30	15.00	26.37	118.67	0.12
Broiler	2	0.158	20	15.80	NA	NA	NA
Laying hens	1.9	0.13	15	10.26	NA	NA	NA
Turkey	7	0.5	15	10.71	NA	NA	NA
Salmon	5	0.03	NA	NA	26.37	15.82	0.02
Carp	1	0.02	NA	NA	NA	NA	NA
Cat	4	0.06	NA	NA	26.37	98.89	0.10
Dog	25	0.36	30	4.32	26.37	71.01	0.07
Horse	450	9	NA	NA	NA	NA	NA

TABLE C.18 Dietary exposure to PAT protein in farm and companion animals, based on the consumption of soybean protein concentrate.

Animal species	Animal body weight	Total daily intake of FEED	Inclusion rate of SOYBEAN protein concentrate	Daily dietary intake of SOYBEAN protein concentrate	NEP level PAT	Daily dietary exposure to NEP	
Category	kg	kg ^{DM} /animal	IR %	g ^{DM} /kg ^{BW}	μg ^{NEP} /g ^{DM}	μg ^{NEP} /kg ^{BW}	mg ^{NEP} /kg ^{BW}
Dairy cow	650	25	10	3.85	NA	NA	NA
Beef cattle	500	12	10	2.40	NA	NA	NA
Dairy Sheep	80	2.8	14	4.90	NA	NA	NA
Dairy Goat	60	3.4	11	6.23	NA	NA	NA
Rabbit	2	0.15	20	15.00	NA	NA	NA
Fattening pig	100	3	20	6.00	NA	NA	NA
Lactating sow	200	6	10	3.00	NA	NA	NA
Piglet	20	1	30	15.00	5.84	26.28	0.026
Broiler	2	0.158	20	15.80	NA	NA	NA
Laying hens	1.9	0.13	15	10.26	NA	NA	NA
Turkey	7	0.5	15	10.71	NA	NA	NA
Salmon	5	0.03	NA	NA	5.84	3.50	0.004
Carp	1	0.02	NA	NA	NA	NA	NA
Cat	4	0.06	NA	NA	5.84	21.90	0.022
Dog	25	0.36	30	4.32	5.84	15.73	0.016
Horse	450	9	NA	NA	NA	NA	NA

TABLE C.19 Dietary exposure to AAD-12 protein in farm and companion animals, based on the consumption of soybean protein concentrate.

Animal species	Animal body weight	Total daily intake of FEED	Inclusion rate of SOYBEAN protein concentrate	Daily dietary intake of SOYBEAN protein concentrate	NEP level AAD-12	Daily dietary exposure to NEP	
Category	kg	kg ^{DM} /animal	IR %	g ^{DM} /kg ^{BW}	μg ^{NEP} /g ^{DM}	μg ^{NEP} /kg ^{BW}	mg ^{NEP} /kg ^{BW}
Dairy cow	650	25	10	3.85	NA	NA	NA
Beef cattle	500	12	10	2.40	NA	NA	NA
Dairy Sheep	80	2.8	14	4.90	NA	NA	NA
Dairy Goat	60	3.4	11	6.23	NA	NA	NA
Rabbit	2	0.15	20	15.00	NA	NA	NA
Fattening pig	100	3	20	6.00	NA	NA	NA
Lactating sow	200	6	10	3.00	NA	NA	NA
Piglet	20	1	30	15.00	138.03	621.14	0.62
Broiler	2	0.158	20	15.80	NA	NA	NA
Laying hens	1.9	0.13	15	10.26	NA	NA	NA
Turkey	7	0.5	15	10.71	NA	NA	NA
Salmon	5	0.03	NA	NA	138.03	82.82	0.08
Carp	1	0.02	NA	NA	NA	NA	NA
Cat	4	0.06	NA	NA	138.03	517.61	0.52
Dog	25	0.36	30	4.32	138.03	371.69	0.37
Horse	450	9	NA	NA	NA	NA	NA

TABLE C.20 Dietary exposure to 2mEPSPS protein in farm and companion animals, based on the consumption of soybean protein concentrate.

Animal species	Animal body weight	Total daily intake of FEED	Inclusion rate of SOYBEAN protein concentrate	Daily dietary intake of SOYBEAN protein concentrate	NEP level 2mEPSPS	Daily dietary exposure to NEP	
Category	kg	kg ^{DM} /animal	IR %	g ^{DM} /kg ^{BW}	μg ^{NEP} /g ^{DM}	μg ^{NEP} /kg ^{BW}	mg ^{NEP} /kg ^{BW}
Dairy cow	650	25	10	3.85	NA	NA	NA
Beef cattle	500	12	10	2.40	NA	NA	NA
Dairy Sheep	80	2.8	14	4.90	NA	NA	NA
Dairy Goat	60	3.4	11	6.23	NA	NA	NA
Rabbit	2	0.15	20	15.00	NA	NA	NA
Fattening pig	100	3	20	6.00	NA	NA	NA
Lactating sow	200	6	10	3.00	NA	NA	NA
Piglet	20	1	30	15.00	7.41	33.35	0.033
Broiler	2	0.158	20	15.80	NA	NA	NA
Laying hens	1.9	0.13	15	10.26	NA	NA	NA
Turkey	7	0.5	15	10.71	NA	NA	NA
Salmon	5	0.03	NA	NA	7.41	4.45	0.004
Carp	1	0.02	NA	NA	NA	NA	NA
Cat	4	0.06	NA	NA	7.41	27.79	0.028
Dog	25	0.36	30	4.32	7.41	19.95	0.020
Horse	450	9	NA	NA	NA	NA	NA

TABLE C.21 Dietary exposure to Cry14Ab-1 protein in farm and companion animals, based on the consumption of soybean forage/silage.

Animal species	Animal body weight	Total daily intake of FEED	Inclusion rate of SOYBEAN forage/silage	Daily dietary intake of SOYBEAN forage/silage	NEP level Cry14Ab-1	Daily dietary exposure to NEP	
Category	kg	kg ^{DM} /animal	IR %	g ^{DM} /kg ^{BW}	μg ^{NEP} /g ^{DM}	μg ^{NEP} /kg ^{BW}	mg ^{NEP} /kg ^{BW}
Dairy cow	650	25	10	3.85	54.73	421.00	0.42
Beef cattle	500	12	10	2.40	NA	NA	NA
Dairy Sheep	80	2.8	14	4.90	NA	NA	NA
Dairy Goat	60	3.4	11	6.23	NA	NA	NA
Rabbit	2	0.15	20	15.00	NA	NA	NA
Fattening pig	100	3	20	6.00	NA	NA	NA
Lactating sow	200	6	10	3.00	NA	NA	NA
Piglet	20	1	30	15.00	NA	NA	NA
Broiler	2	0.158	20	15.80	NA	NA	NA
Laying hens	1.9	0.13	15	10.26	54.73	374.47	0.37
Turkey	7	0.5	15	10.71	NA	NA	NA
Salmon	5	0.03	NA	NA	NA	NA	NA
Carp	1	0.02	NA	NA	NA	NA	NA
Cat	4	0.06	NA	NA	NA	NA	NA
Dog	25	0.36	30	4.32	NA	NA	NA
Horse	450	9	NA	NA	NA	NA	NA

TABLE C.22 Dietary exposure to HPPD-4 protein in farm and companion animals, based on the consumption of soybean forage/silage.

Animal species	Animal body weight	Total daily intake of FEED	Inclusion rate of SOYBEAN forage/silage	Daily dietary intake of SOYBEAN forage/silage	NEP level HPPD-4	Daily dietary exposure to NEP	
Category	kg	kg ^{DM} /animal	IR %	g ^{DM} /kg ^{BW}	μg ^{NEP} /g ^{DM}	μg ^{NEP} /kg ^{BW}	mg ^{NEP} /kg ^{BW}
Dairy cow	650	25	10	3.85	16.31	125.46	0.13
Beef cattle	500	12	10	2.40	NA	NA	NA
Dairy Sheep	80	2.8	14	4.90	NA	NA	NA
Dairy Goat	60	3.4	11	6.23	NA	NA	NA
Rabbit	2	0.15	20	15.00	NA	NA	NA
Fattening pig	100	3	20	6.00	NA	NA	NA
Lactating sow	200	6	10	3.00	NA	NA	NA
Piglet	20	1	30	15.00	NA	NA	NA
Broiler	2	0.158	20	15.80	NA	NA	NA
Laying hens	1.9	0.13	15	10.26	16.31	111.59	0.11
Turkey	7	0.5	15	10.71	NA	NA	NA
Salmon	5	0.03	NA	NA	NA	NA	NA
Carp	1	0.02	NA	NA	NA	NA	NA
Cat	4	0.06	NA	NA	NA	NA	NA
Dog	25	0.36	30	4.32	NA	NA	NA
Horse	450	9	NA	NA	NA	NA	NA

TABLE C.23 Dietary exposure to PAT protein in farm and companion animals, based on the consumption of soybean forage/silage.

Animal species	Animal body weight	Total daily intake of FEED	Inclusion rate of SOYBEAN forage/silage	Daily dietary intake of SOYBEAN forage/silage	NEP level PAT	Daily dietary exposure to NEP	
Category	kg	kg ^{DM} /animal	IR %	g ^{DM} /kg ^{BW}	μg ^{NEP} /g ^{DM}	μg ^{NEP} /kg ^{BW}	mg ^{NEP} /kg ^{BW}
Dairy cow	650	25	10	3.85	6.03	46.38	0.05
Beef cattle	500	12	10	2.40	NA	NA	NA
Dairy Sheep	80	2.8	14	4.90	NA	NA	NA
Dairy Goat	60	3.4	11	6.23	NA	NA	NA
Rabbit	2	0.15	20	15.00	NA	NA	NA
Fattening pig	100	3	20	6.00	NA	NA	NA
Lactating sow	200	6	10	3.00	NA	NA	NA
Piglet	20	1	30	15.00	NA	NA	NA
Broiler	2	0.158	20	15.80	NA	NA	NA
Laying hens	1.9	0.13	15	10.26	6.03	41.26	0.04
Turkey	7	0.5	15	10.71	NA	NA	NA
Salmon	5	0.03	NA	NA	NA	NA	NA
Carp	1	0.02	NA	NA	NA	NA	NA
Cat	4	0.06	NA	NA	NA	NA	NA
Dog	25	0.36	30	4.32	NA	NA	NA
Horse	450	9	NA	NA	NA	NA	NA

TABLE C.24 Dietary exposure to AAD-12 protein in farm and companion animals, based on the consumption of soybean forage/silage.

Animal species	Animal body weight	Total daily intake of FEED	Inclusion rate of SOYBEAN forage/silage	Daily dietary intake of SOYBEAN forage/silage	NEP level AAD-12	Daily dietary exposure to NEP	
Category	kg	kg ^{DM} /animal	IR %	g ^{DM} /kg ^{BW}	μg ^{NEP} /g ^{DM}	μg ^{NEP} /kg ^{BW}	mg ^{NEP} /kg ^{BW}
Dairy cow	650	25	10	3.85	69.22	532.46	0.53
Beef cattle	500	12	10	2.40	NA	NA	NA
Dairy Sheep	80	2.8	14	4.90	NA	NA	NA
Dairy Goat	60	3.4	11	6.23	NA	NA	NA
Rabbit	2	0.15	20	15.00	NA	NA	NA
Fattening pig	100	3	20	6.00	NA	NA	NA
Lactating sow	200	6	10	3.00	NA	NA	NA
Piglet	20	1	30	15.00	NA	NA	NA
Broiler	2	0.158	20	15.80	NA	NA	NA
Laying hens	1.9	0.13	15	10.26	69.22	473.61	0.47
Turkey	7	0.5	15	10.71	NA	NA	NA
Salmon	5	0.03	NA	NA	NA	NA	NA
Carp	1	0.02	NA	NA	NA	NA	NA
Cat	4	0.06	NA	NA	NA	NA	NA
Dog	25	0.36	30	4.32	NA	NA	NA
Horse	450	9	NA	NA	NA	NA	NA

TABLE C.25 Dietary exposure to 2mEPSPS protein in farm and companion animals, based on the consumption of soybean forage/silage.

Animal species	Animal body weight	Total daily intake of FEED	Inclusion rate of SOYBEAN forage/silage	Daily dietary intake of SOYBEAN forage/silage	NEP level 2mEPSPS	Daily dietary exposure to NEP	
Category	kg	kg ^{DM} /animal	IR %	g ^{DM} /kg ^{BW}	µg ^{NEP} /g ^{DM}	µg ^{NEP} /kg ^{BW}	mg ^{NEP} /kg ^{BW}
Dairy cow	650	25	10	3.85	71	546.15	0.55
Beef cattle	500	12	10	2.40	NA	NA	NA
Dairy Sheep	80	2.8	14	4.90	NA	NA	NA
Dairy Goat	60	3.4	11	6.23	NA	NA	NA
Rabbit	2	0.15	20	15.00	NA	NA	NA
Fattening pig	100	3	20	6.00	NA	NA	NA
Lactating sow	200	6	10	3.00	NA	NA	NA
Piglet	20	1	30	15.00	NA	NA	NA
Broiler	2	0.158	20	15.80	NA	NA	NA
Laying hens	1.9	0.13	15	10.26	71	485.79	0.49
Turkey	7	0.5	15	10.71	NA	NA	NA
Salmon	5	0.03	NA	NA	NA	NA	NA
Carp	1	0.02	NA	NA	NA	NA	NA
Cat	4	0.06	NA	NA	NA	NA	NA
Dog	25	0.36	30	4.32	NA	NA	NA
Horse	450	9	NA	NA	NA	NA	NA