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Assessment of genetically modified soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2016-128)

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Abstract

Soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 (four-event stack soybean) was produced by conventional crossing to combine four single events: MON 87751, MON 87701, MON 87708 and MON 89788. The GMO Panel previously assessed the four single events and did not identify safety concerns. No new data on the single events have been identified that would 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 and allergenicity assessment indicate that the combination of the single soybean events and of the newly expressed proteins in the four-event stack soybean does not give rise to food and feed safety and nutritional concerns. The GMO Panel concludes that the four-event stack soybean, as described in this application, is as safe as and nutritionally equivalent to the non-GM comparator and the non-GM reference varieties tested. In the case of accidental release of viable seeds of the four-event stack soybean 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 the four-event stack soybean. Post-market monitoring of food/feed is not considered necessary. The GMO Panel concludes that the four-event stack soybean is as safe as the non-GM comparator and the tested non-GM reference varieties with respect to potential effects on human and animal health and the environment.

Keywords: genetically modified organism (GMO), soybean (*Glycine max*), MON 87751, MON 87701, MON 87708, MON 89788, herbicide tolerant, insect resistant

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Summary

Following the submission of application EFSA-GMO-NL-2016-128 under Regulation (EC) No 1829/2003 from Monsanto (hereafter referred to as 'the applicant'), the Panel on Genetically Modified Organisms of the European Food Safety Authority (hereafter referred to as the 'GMO Panel') was asked to deliver a scientific opinion on genetically modified (GM) soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 (hereafter referred to as 'the four-event stack soybean'). The scope of application EFSA-GMO-NL-2016-128 is for the placing on the market of the four-event stack soybean for food and feed uses, import and processing.

The four-event stack soybean was produced by conventional crossing to combine four single soybean events: MON 87751 (expressing Cry1A.105 and Cry2Ab2), MON 87701 (expressing Cry1Ac), MON 87708 (expressing DMO) and MON 89788 (CP4 EPSPS), to confer tolerance to dicamba- and glyphosate-containing herbicides and resistance against specific lepidopteran pests.

The GMO Panel evaluated the four-event stack soybean with reference to the scope and appropriate principles described in its guidelines for the risk assessment of GM plants and derived food and feed, the environmental risk assessment of GM plants and the post-market environmental monitoring (PMEM) of GM plants. The GMO Panel considered the information available on the single events, the four-event stack soybean, the scientific comments submitted by the Member States and the relevant scientific literature.

The single events MON 87751, MON 87701, MON 87708 and MON 89788 were previously assessed by the European Food Safety Authority (EFSA) and no concerns on their safety were identified. No new safety issue was identified by updated bioinformatic analyses, nor reported by the applicant concerning the four single soybean events, since the publication of the respective scientific opinions. Consequently, the GMO Panel considers that its previous conclusions on the safety of the single soybean events remain valid.

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

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

The comparative analysis of forage and seed composition and agronomic/phenotypic characteristics identified no differences between the four-event stack soybean and the non-GM comparator that required further assessment for food/feed safety or environmental impact, except for the levels of Gly m 4 protein in seeds. Those changes were further assessed and not found to have a safety impact.

The molecular characterisation, the comparative analysis and the outcome of the toxicological and allergenicity assessment indicate that the combination of the single soybean events and of the newly expressed proteins in the four-event stack soybean does not give rise to food and feed safety and nutritional concerns. The GMO Panel concludes that soybean MON 87751 × MON 87701 × MON 87708 × MON 89788, as described in this application, is as safe as and nutritionally equivalent to the non-GM comparator and the commercial non-GM soybean reference varieties (hereafter 'non-GM reference varieties') tested.

Considering the combined events and their potential interactions, the outcome of the comparative analysis and the routes and levels of exposure, the GMO Panel concludes that soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 would not raise safety concerns in the case of accidental release of viable GM soybean seeds into the environment.

Given the absence of safety concerns for foods and feeds from the four-event stack soybean, the GMO Panel considers that post-market monitoring of food/feed is not considered necessary. The PMEM plan and reporting intervals are in line with the intended uses of the four-event stack soybean. The literature searches did not identify any relevant publications on the four-event stack soybean. In the context of annual PMEM reports, the applicant could further improve future literature searches according to the GMO Panel recommendations provided in this scientific opinion.

The GMO Panel concludes that soybean MON 87751 × MON 87701 × MON 87708 × MON 89788, as described in this application, is as safe as the non-GM comparator and the tested non-GM reference varieties with respect to potential effects on human and animal health and the environment.

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1. Introduction

The scope of application EFSA-GMO-NL-2016-128 is for food and feed uses, import and processing in the European Union (EU) of the genetically modified (GM) insect resistant and herbicide tolerant soybean MON 87751 × MON 87701 × MON 87708 × MON 89788.

1.1. Background

On 23 December 2015, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands the application EFSA-GMO-NL-2016-128 for authorisation of soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 (Unique Identifier MON-87751-7 × MON 877Ø1-2 × MON-877Ø8-9 × MON-89788-1), submitted by Monsanto (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003.[1]

Following receipt of application EFSA-GMO-NL-2016-128, EFSA informed EU Member States and the European Commission, and made the summary of the application available to the public on the EFSA website.[2]

EFSA checked the application for compliance with the relevant requirements of Regulation (EC) No 1829/2003 and Regulation (EU) No 503/2013[3] and, when needed, asked the applicant to supplement the initial application. On 22 August 2016, EFSA declared the application valid, and made the valid application available to EU Member States and the European Commission.

From the validity date, EFSA and its scientific Panel on Genetically Modified Organisms (hereafter referred to as the 'GMO Panel') endeavoured to respect a time limit of six months to issue a scientific opinion on application EFSA-GMO-NL-2016-128. Such time limit was extended whenever EFSA and/or its GMO Panel requested supplementary information to the applicant. According to Regulation (EC) No 1829/2003, any supplementary information provided by the applicant during the risk assessment was made available to the EU Member States and European Commission (for further details, see the section 'Documentation', below).

In accordance with Regulation (EC) No 1829/2003, EFSA consulted the nominated risk assessment bodies of EU Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC.[4] The EU Member States had three months to make their opinion known on the application EFSA-GMO-NL-2016-128 as of date of validity.

1.2. Terms of Reference as provided by the requestor

According to Articles 6 and 18 of Regulation (EC) No 1829/2003, EFSA and its GMO Panel were requested to carry out a scientific risk assessment of soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 in the context of its scope as defined in application EFSA-GMO-NL-2016-128.

According to Regulation (EC) No 1829/2003, this scientific opinion is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation, including the opinions of the nominated risk assessment bodies of EU Member States.[5]

In addition to the present scientific opinion on soybean MON 87751 × MON 87701 × MON 87708 × MON 89788, EFSA and its GMO Panel were also asked to report on the particulars listed under Articles 6(5) and 18(5) of Regulation (EC) No 1829/2003. The relevant information is made available in the EFSA Register of Questions,[6] including: the information required under Annex II to the Cartagena Protocol, a labelling proposal and a post-market environmental Monitoring (PMEM) plan as provided by the applicant; the method(s), validated by the Community reference laboratory, for detection, including sampling and identification of the transformation event in the food-feed and/or foods-feeds produced from it; and the appropriate reference materials.

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.

[2] Available online at the EFSA Register of Questions: http://registerofquestions.efsa.europa.eu/roqFrontend/login?0) querying the assigned Question number.

[3] Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. OJ L157, 8.6.2013, p. 1–48.

[4] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 12.3.2001, p. 1–38.

[5] Opinions of the nominated risk assessment bodies of EU Member States can be found at the EFSA Register of Questions (http://registerofquestions.efsa.europa.eu/roqFrontend/login?0) querying the assigned Question number.

[6] http://registerofquestions.efsa.europa.eu/roqFrontend/login?0 querying the assigned Question number.

2. Data and methodologies

2.1. Data

The GMO Panel based its scientific risk assessment of soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 on the valid application EFSA-GMO-NL-2016-128, additional information provided by the applicant during the risk assessment, scientific comments submitted by EU Member States and relevant peer-reviewed scientific publications. As part of this comprehensive information package, the GMO Panel received additional unpublished studies submitted by the applicant in order to comply with the specific provisions of Regulation (EU) No 503/2013. A list of these additional unpublished studies is provided in Appendix B.

2.2. Methodologies

The GMO Panel conducted its assessment in line with the principles described in Regulation (EU) No 503/2013, its applicable guidelines (EFSA GMO Panel, 2010a,b, 2011a,b, 2015), explanatory notes and statements (EFSA, 2014, 2017a,b, 2019) for the risk assessment of GM plants. During its risk assessment, the GMO Panel considered all additional unpublished studies as listed in Appendix B for potential effects on human and animal health and the environment.

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

The GMO Panel also assessed the applicant's literature searches, which include a scoping review, in accordance with the recommendations on literature searching outlined in EFSA (2010, 2017a, 2019).

In the frame of the contracts OC/EFSA/GMO/2013/01 and OC/EFSA/GMO/2014/01, contractors performed preparatory work and delivered reports on the methods applied by the applicant in performing bioinformatic and statistical analyses, respectively.

3. Assessment

3.1. Introduction

Application EFSA-GMO-NL-2016-128 covers the four-event stack soybean MON 87751 × MON 87701 × MON 87708 × MON 89788. This four-event stack soybean was developed by conventional crossing to combine four single events: MON 87751 (expressing the Cry1A.105 and Cry2Ab2 proteins for protection against specific lepidopteran pests), MON 87701 (expressing the Cry1Ac protein for resistance against specific lepidopteran pests), MON 87708 (expressing the DMO proteins for tolerance to dicamba-containing herbicides) and MON 89788 (expressing the CP4 EPSPS protein for tolerance to glyphosate-containing herbicides). It should be noted that the assessment of herbicide residues relevant for this application has been investigated by the EFSA Pesticides Unit (EFSA, 2013, 2018a).

All four single events were assessed previously (see Table 1) and no concerns for human and animal health or environmental safety were identified.

Table 1: Single soybean events assessed by the GMO Panel

Event	Application or mandate	EFSA Scientific Opinion
MON 87751	EFSA-GMO-NL-2014-121	EFSA GMO Panel (2018a)
MON 87701	EFSA-GMO-BE-2010-79	EFSA GMO Panel (2011b)
MON 87708	EFSA-GMO-NL-2011-93	EFSA GMO Panel (2013)
MON 89788	EFSA-GMO-NL-2006-36	EFSA (2008)
	EFSA-GMO-RX-011	EFSA GMO Panel (2018b)

3.2. Updated information on single events[7,8]

Since the publication of the scientific opinions on the single soybean events (see Table 1), no safety issue concerning the four single events has been reported by the applicant.

Updated bioinformatic analyses for soybean events MON 87751, MON 87701, MON 87708 and MON 89788 confirmed that no known endogenous genes were disrupted by any of the inserts.

Updated bioinformatic analyses of the amino acid sequence of the newly expressed Cry1A.105, Cry2Ab2, Cry1Ac, DMO and CP4 EPSPS proteins confirmed previous results indicating no significant similarities to toxins and allergens. 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 soybean events MON 87751, MON 87701, MON 87708 and MON 89788 confirmed previous analyses (Table 1). These analyses indicate that the production of a new peptide showing significant similarities to toxins or allergens for any of the events in soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 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 for soybean events MON 87751, MON 87701, MON 87708 and MON 89788 to microbial DNA. The likelihood and potential consequences of plant-to-bacteria gene transfer are described in Section 3.7.2.1.

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

3.3. Systematic literature review[9]

The GMO Panel assessed the applicant's literature searches on soybean MON 87751 × MON 87701 × MON 87708 × MON 89788, which include a scoping review, according to the guidelines given in EFSA (2010, 2017a, 2019).

A systematic review as referred to in Regulation (EU) No 503/2013 has not been provided in support to the risk assessment of the application EFSA-GMO-NL-2016-128. Based on the outcome of the scoping review, the GMO Panel agrees that there is limited value of undertaking a systematic review for soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 at present.

Although the overall quality of the performed literature searches is acceptable, the GMO Panel considers that future searches on soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 could be improved. The GMO Panel therefore recommends the applicant to:

- Ensure that enough search term variation is used (covering possible synonyms, related terms, acronyms, spelling variants, old and new terminology, brand and generic names, lay and scientific terminology, common typos, translation issues);
- Ensure that enough truncation is used and that it is being used consistently;
- Include controlled vocabulary (subject indexing) in the searches when available, and where subject headings are available, use both free-text terms and controlled vocabulary in the searches.

The literature searches did not identify any relevant publications on soybean MON 87751 × MON 87701 × MON 87708 × MON 89788.

3.4. Molecular characterisation

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

3.4.1. Genetic elements and their biological function[7]

Soybean events MON 87751, MON 87701, MON 87708 and MON 89788 were combined by conventional crossing to produce the four-event stack soybean MON 87751 × MON 87701 × MON 87708 × MON 89788. The structure of the inserts introduced into the four-event stack soybean is described in detail in the respective EFSA scientific opinions (Table 1) and no new genetic

[7] Dossier: Part II – Section 1.2.2.2
[8] Additional information: 13/8/2018 and 3/10/2018.
[9] Dossier: Part II – Section 7; additional information: 20/7/2018.

modifications were involved. Genetic elements in the expression cassettes of the single events are summarised in Table 2.

Intended effects of the inserts in soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 are summarised in Table 3.

Based on the known biological function of the newly expressed proteins (Table 3), the only potential functional interactions at the biological level are between the three Cry proteins in susceptible insects.

Table 2: Genetic elements in the expression cassettes of the events stacked in soybean MON 87751 × MON 87701 × MON 87708 × MON 89788

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
MON 87751	*act2 (A. thaliana)* *rbcS4* (*A. thaliana*)	– –	*CTP2 (A. thaliana)* *rbcS4* (*A. thaliana*)	*cry2Ab2** (*B. thuringiensis*) *cry1A.105** (*B. thuringiensis*)	*mt* (*O. sativa*) *pt1* (*M. truncatula*)
MON 87701	*rbcS4* (*A. thaliana*)	–	*CTP1 (A. thaliana)*	*Cry1Ac** (*B. thuringiensis*)	*7Sα′* (*Glycine max*)
MON 87708	*PC1SV* (Peanut chlorotic streak caulimovirus)	*TEV* (Tobacco Etch virus)	*rbcS* (*Pisum sativum*)	*dmo* (*Stenotrophomonas maltophilia*)	*E9* (*Pisum sativum*)
MON 89788	*FMV/EF-1a* (Figwort Mosaic Virus and *A. thaliana*)	L–*EF-1a*/I–*EF-1a* (*A. thaliana*)	*CTP2 (A. thaliana)*	*cp4 epsps** (*A.* tumefaciens CP4)	*E9* (*Pisum sativum*)

*: Codon optimised.
–: When no element was specifically introduced to optimise expression.

Table 3: Characteristics and intended effects of the events stacked in soybean MON 87751 × MON 87701 × MON 87708 × MON 89788

Event	Protein	Donor organism and biological function	Intended effects in GM plant
MON 87751	Cry2Ab2 Cry1A.105	Based on genes from *B. thuringiensis* subsp. *kurstaki*. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes (Widner and Whiteley, 1990)	Event MON 87751 expresses the Cry2Ab2, a protein toxic to certain lepidopteran larvae feeding on soybean
		Based on genes from *B. thuringiensis. B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes (Widner and Whiteley, 1990)	Event MON 87751 expresses a modified version of the Cry1A-type protein. Cry1A.105 is a protein toxic to certain lepidopteran larvae feeding on soybean
MON 87701	Cry1Ac	Based on genes from *B. thuringiensis* subsp. *kurstaki* HD-73. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes (Donovan et al., 1992)	Event MON 87701 expresses a chimeric, truncated *cry1Ac* gene which was modified to enhance its expression in plants. Cry1Ac is a chimeric protein toxic to certain lepidopteran larvae feeding on soybean
MON 87708	DMO	Based on a gene from *Stenotrophomonas maltophilia* strain DI-6. Dicamba mono-oxygenase (DMO) is an enzyme that catalyses the demethylation of dicamba to the non-herbicidal compound 3,6-dichlorosalicylic acid and formaldehyde (Herman et al., 2005)	Event MON 87708 expresses DMO protein which degrades the herbicide dicamba and thus confers tolerance to this herbicide

Event	Protein	Donor organism and biological function	Intended effects in GM plant
MON 89788	CP4 EPSPS	Based on a gene from *Agrobacterium* strain CP4 (Barry et al., 2001). 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) is an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Herrmann, 1995)	Event MON 89788 expresses the bacterial CP4 EPSPS protein which confers tolerance to glyphosate-containing herbicides as it has lower affinity towards glyphosate than the plant endogenous enzyme

3.4.2. Integrity of the events in the four-event stack[10]

The genetic stability of the inserted DNA over multiple generations in the single soybean events MON 87751, MON 87701, MON 87708 and MON 89788 was demonstrated previously (see Table 1). Integrity of these genetically independent events in soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 was demonstrated by polymerase chain reaction (PCR) and sequence analysis, which show that the sequences of the events (inserts and their flanking regions) in the four-event stack soybean are identical to the sequences originally reported for the four single events.

3.4.3. Information on the expression of the inserts[11,12]

Cry2Ab2, Cry1A.105, Cry1Ac, DMO and CP4 EPSPS protein levels were analysed by enzyme-linked immunosorbent assay (ELISA) in material harvested from a field trial at five locations in the USA in 2013. Samples analysed included over season leaf (OSL1-4), root (R6), forage (R6) and seed (R8) treated with intended herbicides. In order to assess the changes in protein expression levels which may result from potential interactions between the events, protein levels were determined for the four-event stack soybean and the corresponding single events in different parts of the plant.

The levels of all the newly expressed proteins in the four-event stack soybean were comparable to those of the single events (Appendix A). Therefore, there is no indication of an interaction that may affect the levels of the newly expressed proteins in this stack.

3.4.4. Conclusions of the molecular characterisation

The molecular data establish that the events stacked in soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins are comparable in the four-event stack and in the 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 function of the newly expressed proteins, the only potential functional interactions are between the three Cry proteins in susceptible insects, which will be addressed in Section 3.7.

3.5. Comparative analysis[13]

3.5.1. Overview of studies conducted for the comparative analysis

Application EFSA-GMO-NL-2016-128 presents data on agronomic and phenotypic characteristics as well as on forage and seed composition of soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 (Table 4).

[10] Dossier: Part II – Section 1.2.2.2.a and additional study MSL0026332.
[11] Dossier: Part II – Section 1.2.2.3 and additional study MSL0027230.
[12] Additional information: 14/11/2018, 25/2/2019 and 8/5/2019.
[13] Dossier: Part II – Section 1.3; additional information: 13/8/2018 and 29/1/2019.

Table 4: Overview of comparative analysis studies to characterise the four-event stack soybean in application EFSA-GMO-NL-2016-128

Study focus	Study details	Comparator	Non-GM reference varieties
Agronomic and phenotypic characteristics	Field trials, 2013 and 2016, US, 11 sites[(a)]	A3555	31[(b)]
Compositional analysis	Field trials, 2013, US, 9 sites[(a)]		18[(b)]

(a): Eight field trials conducted in 2013 were used for both the compositional and the agronomic/phenotypic analysis: at Jackson County, Arkansas; Jefferson County, Iowa; Champaign County, Illinois; Pawnee County, Kansas; Perquimans County, North Carolina; Polk County, Nebraska; Miami County, Ohio; and Lehigh County, Pennsylvania. A field trial in Stark County, Illinois, 2013, was used only for compositional analysis. Four field trials conducted in 2016 were used only for the agronomic/phenotypic analysis: at Jasper County, Iowa; Clinton County, Illinois; Clinton County, Indiana; and Lehigh County, Pennsylvania (the latter was tested both in 2013 and in 2016).

(b): The following 18 varieties were used for both the compositional and the agronomic/phenotypic characterisation: A3244, A3525, C3211N, C3884N, DWIGHT, eMerge 348TC, Garst 3585N, Hoffman H419, Hoffman HS387, Lewis 372, Maverick, Midland 363, NE3202, NuPride 2954, Steward SB3454, Stine 3300-0, Wilken 3316 and WILLIAMS 82. In addition, the field trials used for agronomic/phenotypic analysis included the following 13 varieties: Asgrow A3253, Asgrow A3956, Becks 331N, Becks 389N, Great Lakes GL3029, ILLINI 2880A, ILLINI 3477N, ILLINI 3880B, ILLINI 6336N, Legend Seeds LS 2880NHP, Stine 3120-2, Stine 3520-2 and Stine 3900-2.

3.5.2. Experimental field trial design and statistical analysis

At each field trial site, the following materials were grown: soybean MON 87751 × MON 87701 × MON 87708 × MON 89788, the comparator soybean A3555 and four non-GM reference varieties. All materials were treated with conventional herbicides management regimes; in addition, the field trials included soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 exposed to the intended dicamba- and glyphosate-containing herbicides on top of the conventional herbicides.

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 soybean MON 87751 × MON 87701 × MON 87708 × MON 89788, the application of a difference test (between the GM soybean and its comparator) and an equivalence test (between the GM soybean and the set of non-GM reference varieties).[14] The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).[15]

3.5.3. Suitability of selected test materials

3.5.3.1. Selection of the GM soybean line and comparator

To obtain the four-event stack GM soybean, the single event MON 87751 and the three-event stack MON 87701 × MON 87708 × MON 89788 were combined by conventional crossing in the non-GM soybean varieties A3555 and A3525, respectively, before crossing.

The comparator used in the field trials is the non-GM soybean variety A3555, which has high similarity with the soybean line A3525. As documented by the pedigree and by the additional information, the EFSA GMO Panel considers the selected line (A3555) a suitable comparator for the comparative analysis.

The GM soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 and its comparator A3555 both belong to the maturity group 3.5 that is appropriate for growing in a range of environments across North America.

3.5.3.2. Selection of commercial non-GM soybean reference varieties

Commercial non-GM soybean reference varieties with maturity groups ranging from 2.8 to 4.1 were included in the field trials. Based on the information on the maturity groups, the GMO Panel considers that the selected non-GM soybean reference varieties are appropriate for the comparative analysis.

[14] The purpose of the test of equivalence is to evaluate the estimated mean values for the stack GM crop taking into account natural variability as defined by a set of non-GM reference varieties with a history of safe use for consumption as food or feed.

[15] In detail, the four outcomes are: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence).

3.5.3.3. Seed production and quality

The seeds of soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 and the comparator used in the field trials (see Table 4) were produced, harvested and stored under similar conditions before being sown. The seed lots were verified for their purity via event specific quantitative PCR analysis. The mean germination rates of soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 and the non-GM comparator were 99% and 99%, respectively (seed lots used in the 2013 field trials) and 99% and 98%, respectively (seed lots used in the 2016 field trials). The GMO Panel considers that the starting seed used as test material in the agronomic, phenotypic and compositional studies was of suitable quality.

3.5.3.4. Conclusion on suitability

The GMO Panel is of the opinion that the soybean MON 87751 × MON 87701 × MON 87708 × MON 89788, its comparator and the non-GM soybean reference varieties were properly selected and are of acceptable quality. Therefore, the test materials are considered suitable for the comparative analysis.

3.5.4. Representativeness of the receiving environments

3.5.4.1. Selection of field trial sites

The selected field trial sites were located in commercial soybean-growing regions of North America. The soil characteristics of the selected fields were diverse,[16] corresponding to optimal, near-optimal and suboptimal conditions for soybean cultivation (Sys et al., 1993). The GMO Panel considers that the selected sites reflect commercial soybean-growing regions in which the test materials are likely to be grown.

3.5.4.2. Meteorological conditions

Maximum and minimum mean temperatures and sum of precipitations were provided on a monthly basis. No exceptional weather conditions were reported at any of the selected field trial sites. The GMO Panel considers that the meteorological data set falls within the range of climatic conditions normally occurring at these sites.

3.5.4.3. Management practices

The field trial included plots containing the four-event stack soybean, plots with the comparator and plots with non-GM reference varieties, all managed according to local agricultural practices. In addition, the field trials included plots containing the four-event stack soybean managed following the same agricultural practices, plus exposed to the intended herbicides. Dicamba- containing herbicide was applied at the V2-V5 growth stage and glyphosate-containing herbicide at V4-R1 growth stage. The GMO Panel considers that the management practices including planting, harvesting and application of plant protection products were appropriate.

3.5.4.4. Conclusion on representativeness

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

3.5.5. Agronomic and phenotypic analysis

Nine agronomic and phenotypic endpoints[17] plus information on abiotic stressors, disease incidence and arthropod damage were evaluated in the field trials (see Table 4). The endpoint pod shattering was not analysed with formal statistical methods because of lack of variability in the data. The remaining eight endpoints were analysed with the tests of difference and equivalence, with the following results:

[16] Soil types of the field trials were silty clay loam, loam, silt loam, loam and sandy loam; soil organic carbon ranged from 0.9% to 6.5%.

[17] Early stand count, days to 50% flowering, plant lodging, plant height, pod shattering, final stand count, seed moisture, 100 seed weight and yield.

- For soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 (treated and not treated), statistically significant differences with the non-GM comparator were identified for two endpoints (100 seed weight and seed moisture), which fell under equivalence category I or II.

3.5.6. Compositional analysis

Soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 seeds and forage harvested from the field trials in 2013 (Table 4) were analysed for 74 different constituents (7 in forage and 67 in seeds), including the key constituents recommended by the OECD (OECD, 2012). For 14 fatty acids in seed,[18] more than 50% of the observations were below the limit of quantification. The statistical analysis was applied to the remaining 60 constituents (7 in forage[19] and 53 in seed[20]); a summary of the outcome of the test of difference and the test of equivalence is presented in Table 5.

- For soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 (not treated), statistically significant differences with the non-GM comparator were identified for 25 endpoints (one in forage and 24 in seed) which all fell under equivalence category I or II except for Gly m 4 levels in seed (Table 6).
- For soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 (treated), significant differences with the non-GM comparator were identified for 17 endpoints (one in forage and 16 in seed) which all fell under equivalence category I or II.

Table 5: Outcome of the comparative compositional analysis of soybean MON 87751 × MON 87701 × MON 87708 × MON 89788. The table shows the number of endpoints in each category

		Test of difference[(a)]			
		Not treated[(c)]		Treated[(c)]	
		Not different	Significantly different	Not different	Significantly different
Test of equivalence[(b)]	Category I/II	34	24[(d)]	42	17[(d)]
	Category III/IV	–	1[(e)]	–	–
	Not categorised	1[(f)]	0	1[(f)]	0
	Total endpoints	60		60	

(a): Comparison between soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 and its non-GM comparator.
(b): Four different outcomes: category I (indicating equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence). Not categorised means that the test of equivalence was not applied because of the lack of variation among the non-GM reference varieties.
(c): Treated/not treated with intended herbicides: dicamba and glyphosate (see Section 3.5.4.3).
(d): Endpoints with significant differences between soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 and its non-GM comparator falling in equivalence category I-II. In forage, for both treated and not treated GM: protein. In seed, for both treated and not treated GM: Gly m Bd 30k, glycine, stachyose, palmitic acid (16:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), daidzein, genistein, carbohydrates, moisture, total fat and vitamin E; for the non-treated GM only: arginine, isoleucine, leucine, methionine, phenylalanine, proline, threonine, valine, calcium, ash; for the treated GM only: Gly m 4, glutamic acid and serine.
(e): Gly m 4 level in seed was significantly different between soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 (not treated) and its non-GM comparator and fell under equivalence category III. Quantitative results for this endpoint are reported in Table 6.
(f): Endpoints not categorised for equivalence and without significant differences between the four-event stack soybean (both treated and not treated) and its non-GM comparator: Gly m 1 in seed.

[18] Caprylic acid (8:0), capric acid (10:0), lauric acid (12:0), myristic acid (14:0), myristoleic acid (14:1), pentadecanoic acid (15:0), pentadecenoic acid (15:1), palmitoleic acid (16:1), heptadecanoic acid (17:0), heptadecenoic acid (17:1), γ-linolenic acid (18:3), eicosadienoic acid (20:2), eicosatrienoic acid (20:3) and arachidonic acid (20:4).
[19] Crude protein, crude fat, ash, moisture, carbohydrates by calculation, acid detergent fibre (ADF) and neutral detergent fibre (NDF).
[20] Ash, carbohydrates, moisture, protein, total fat, acid detergent fibre (ADF), neutral detergent fibre (NDF), calcium, phosphorus, alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), arachidic acid (20:0), eicosenoic acid (20:1), behenic acid (22:0), vitamin E, vitamin K1, 2S albumin, Gly m 1, Gly m 3, Gly m 4, Gly m Bd 28k, Gly m Bd 30k, glycinin, ß-conglycinin, phytic acid, raffinose, soybean lectin, stachyose, trypsin inhibitor, daidzein, genistein and glycitein.

The GMO Panel assessed all significant differences between soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 and the non-GM comparator, taking into account potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties. Quantitative results for the endpoint showing significant differences between soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 and the non-GM comparator and falling under equivalence category III are given in Table 6.

Table 6: Quantitative results (estimated means and equivalence limits) for the seed endpoint with significant differences between soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 and its non-GM comparator, falling under category III in the test of equivalence

Endpoint	Soybean MON 87751 × MON 87701 × MON 87708 × MON 89788		Non-GM comparator	Non-GM reference varieties	
	Not treated	Treated[(a)]		Mean	Equivalence limits
Gly m 4 (μg/g fw)	82.51*	92.18*	108.47	133.74	(89.90, 177.58)

fw: fresh weight.
(a): Treated with dicamba- and glyphosate-containing herbicides as described in Section 3.5.4.3.
For soybean MON 87751 × MON 87701 × MON 87708 × MON 89788, significantly different values are marked with an asterisk, while the outcomes of the test of equivalence are differentiated by greyscale backgrounds: white (equivalence category II) and light grey (equivalence category III).

3.5.7. Conclusion of 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 the agronomic and phenotypic characteristics tested between soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 and the non-GM comparator needs further assessment regarding their potential environmental impact.
- None of the compositional differences identified between soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 and the non-GM comparator needs further assessment for food/feed safety except for Gly m 4 levels in seed, which are further discussed in Section 3.6.4.2.

3.6. Food/feed safety assessment

3.6.1. Effects of processing

Soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 will undergo existing production processes used for conventional soybean. Considering the changes observed in the compositional comparative analysis (Section 3.5.6), the processing of the four-event stack soybean into food and feed products is not expected to result in products being different from those of conventional non-GM soybean varieties.

3.6.2. Influence of temperature and pH on newly expressed proteins

Effects of temperature and pH on newly expressed proteins Cry1A.105, Cry2Ab2, Cry1Ac, DMO and CP4 EPSPS have been previously evaluated by the GMO Panel (Table 1). Additional studies on the effects of heat treatment provided for DMO and Cry1Ac were assessed by the GMO panel (Appendix B).

3.6.3. Toxicology

3.6.3.1. Testing of newly expressed proteins

Five proteins (Cry1A.105, Cry2Ab2, Cry1Ac, DMO and CP4 EPSPS) are newly expressed in soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 (Section 3.4.1). The GMO Panel has previously assessed these proteins in the context of the single events (Table 1), and no safety concerns were identified for humans and animals. The unpublished toxicological studies provided in the context of this application (see Appendix B) did not change this conclusion. The GMO Panel is not aware of any other new information that would change this conclusion.

The potential for a functional interaction between the proteins newly expressed in soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 has been assessed with regard to human and animal health. The three insecticidal proteins (Cry1A.105, and Cry2Ab2 and Cry1Ac) are delta-endotoxins acting through cellular receptors found in target insect species. It is reported that the gastrointestinal tract of mammals, including humans, lacks receptors with high specific affinity to Cry proteins (Hammond et al., 2013; Koch et al., 2015). The DMO and CP4 EPSPS proteins are enzymes that catalyse distinct biochemical reactions and act on unrelated substrates with high substrate specificity.

On the basis of the known biological function of the individual newly expressed proteins (Table 3), there is currently no expectation for possible interactions relevant to the food and feed safety of soybean MON 87751 × MON 87701 × MON 87708 × MON 89788.

In vitro protein degradation studies on Cry1A.105, Cry2Ab2, Cry1Ac, DMO and CP4 EPSPS proteins have been previously evaluated by the EFSA GMO Panel and no indications of safety concerns were identified (Table 1).

The GMO Panel concludes that there are no safety concerns to human and animal health related to the newly expressed proteins Cry1A.105, Cry2Ab2, Cry1Ac, DMO and CP4 EPSPS in soybean MON 87751 × MON 87701 × MON 87708 × MON 89788.

3.6.3.2. Testing of new constituents other than newly expressed proteins

No new constituents other than newly expressed proteins have been identified in soybean MON 87751 × MON 87701 × MON 87708 × MON 89788. Therefore, no further food and feed safety assessment of components other than the newly expressed proteins is required.

3.6.3.3. Information on altered levels of food and feed constituents

Gly m 4 levels in seed were significantly different in soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 when compared with its non-GM comparator and showed a lack of equivalence with the non-GM reference varieties (Section 3.5.6). No toxicological concern is identified regarding this compositional change. Further information on safety is provided in Section 3.6.4.2.

3.6.3.4. Testing of the whole genetically modified food and feed

Based on the outcome of the molecular characterisation, comparative analysis and toxicological assessment, no indication of findings relevant to food/feed safety related to the stability and expression of the inserts or to interaction between the transformation events, and no modifications of toxicological concern in the composition of soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 have been identified (see Sections 3.4.4, 3.5.7 and 3.6.3.3). Therefore, animal studies on food/feed derived from soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 are not necessary (EFSA GMO Panel, 2011a).

In accordance to Regulation (EU) No 503/2013, the applicant provided a 90-day oral repeated-dose toxicity study in rats on whole food and feed from each of the single-event soybean MON 87751, MON 87701, MON87708 and MON89788. The four studies had already been provided in the context of the single-event applications and assessed by the GMO Panel (Table 1); no adverse effects related to the administration of the respective GM diets had been identified. In the context of the assessment of soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 and in order to fulfil the requirements of Regulation (EU) No 503/2013 for 90-day studies, upon EFSA's request, the applicant provided additional information on the 90-day studies on the whole food and feed from MON 87708 and MON87701 and a new study on the whole food and feed from MON 89788.

The GMO Panel has previously assessed the above-mentioned additional information on MON 87708 and the new study on MON 89788 in the context of another application under Regulation (EU) 503/2013 (EFSA GMO Panel, 2019a). The additional histopathology[21] provided for the 90-day study on the whole food and feed from MON 87701 showed only sporadic histopathological findings compatible with the spontaneous background pathology of rats of this strain and age.

On the basis of the additional information received, the GMO Panel concludes that all the above-mentioned studies are in line with the requirements of Regulation (EU) 503/2013 and that there are no

[21] Aorta, bone (sternum) with bone marrow, caecum, eyes with optic nerves, lungs, mandibular lymph node, mammary gland (females only), oesophagus, pituitary, prostate, salivary gland, seminal vesicles, skeletal muscle, skin, trachea, urinary bladder, vagina and Peyer's Patches from all animals given the control and 30% test diet.

indications of adverse effects related to the 90-day administration to rats of diets including up to 30% seeds from soybean MON87751, MON87701, MON87708 and MON 897988.

3.6.4. Allergenicity

For the allergenicity assessment, a weight-of-evidence approach was followed, taking into account all the information obtained on the newly expressed proteins, as no single piece of information or experimental method yields sufficient evidence to predict allergenicity (Codex Alimentarius, 2009; EFSA GMO Panel, 2011a; Commission Regulation (EU) No 503/2013). In addition, when known functional aspects of the newly expressed protein or structural similarity to known adjuvants may indicate an adjuvant activity, the possible role of these proteins as adjuvants is considered. When newly expressed proteins with a potential adjuvant activity are expressed together, possible interactions increasing adjuvanticity and impacting the allergenicity of the GM crop are assessed. Furthermore, an assessment of specific newly expressed proteins in relation to their potential to cause coeliac disease was also performed (EFSA GMO Panel, 2017).

3.6.4.1. Assessment of allergenicity of newly expressed proteins

For allergenicity, the GMO Panel has previously evaluated the safety of Cry1A.105, Cry2Ab2, Cry1Ac, DMO and CP4 EPSPS proteins individually, and no concerns on allergenicity were identified in the context of the applications assessed (Table 1). EFSA has recently published a technical report on the safety assessment of genetically modified crops with Cry1Ac confirming previous EFSA opinions (EFSA et al., 2018b). No new information on allergenicity of the proteins newly expressed in this four-event stack soybean that might change the previous conclusions of the GMO Panel has become available. Based on the current knowledge, and as none of the newly expressed proteins showed allergenicity, no reasons for concerns on allergenicity regarding the simultaneous presence of these newly expressed proteins in the four-event stack soybean affecting their allergenicity are expected.

For adjuvanticity, the Bt protein Cry1Ac has been suggested to possess adjuvant activity based on animal studies when applied at relatively high doses (e.g. Vazquez et al., 1999; Santos-Vigil et al., 2018). The Panel has previously evaluated the safety of Cry1A.105, Cry2Ab2 and Cry1Ac proteins, and no concerns on adjuvanticity were identified in the context of the applications assessed (see Table 1). More recently, this aspect has been discussed in detail by EFSA (EFSA et al., 2018b; Parenti et al., 2019). The levels of the individual Bt proteins in the four-event stack soybean are comparable to those in the respective single soybean events (see Section 3.4.3). From the limited experimental evidence available, the GMO Panel did not find indications that the Bt proteins at the levels expressed in this four-event stack soybean might act as adjuvants with the potential to enhance a specific immunoglobulin E (IgE) response and to favour the development of an allergic reaction.

The applicant provided spontaneous information on the safety of the Cry1A.105, Cry2Ab2 and Cry1Ac proteins regarding their potential hazard to cause a coeliac disease response.[22] [23] For such assessment, the applicant followed the principles described in the EFSA GMO Panel guidance document (EFSA GMO Panel, 2017). The assessment of the Cry2Ab2 and Cry1Ac identified no perfect or relevant partial matches with known coeliac disease peptide sequences. The assessment of the Cry1A.105 revealed partial matches which have been previously evaluated by the GMO Panel (EFSA GMO Panel, 2019b). Therefore, no indications of safety concerns were identified by the GMO Panel.

3.6.4.2. Assessment of allergenicity of the GM plant products

Soybean is considered a common allergenic food[24] (OECD, 2012). Therefore, any potential change in the endogenous allergenicity of the GM plant should be assessed (Regulation (EU) No 503/2013). For such assessment, the applicant included in the comparative analysis specific allergens relevant for soybean (Section 3.5.6) measured by specific ELISA methods, which have been previously considered acceptable (EFSA GMO Panel, 2010b; Fernandez et al., 2013; Selb et al., 2017). The applicant also referred to the Kunitz trypsin inhibitor as a potential soybean allergen, which is an anti-nutrient and as

[22] It is pointed out that the requirements laid down in the recent EFSA guidance on allergenicity (EFSA GMO Panel, 2017) are not applicable to this dossier, as described in Section 1.5 'Transition period' of the guidance document.
[23] Additional information: 13/8/2018, 6/5/2019, 17/5/2019, 4/6/2019 and 7/8/2019.
[24] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

such it is already assessed in the compositional analysis (Section 3.5.6). These allergens were selected based on the list of potential soybean allergens described in the pertinent OECD document (OECD, 2012) and a scientific rational supporting their selection was provided by the applicant and considered acceptable by the GMO Panel.

Allergen Gly m 4 levels in soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 (not treated) were significantly different from those of the non-GM comparator and fell under equivalence category III (see Section 3.5.6). For the assessment, the GMO Panel takes into account the fact that the difference reported for this allergen consists in a decrease and that no relevant differences in the content of other allergens were observed. Based on these considerations, no changes in the levels of endogenous allergens raising concern are 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 the four-event stack soybean when compared with that of the non-GM comparator and the non-GM reference varieties tested.

3.6.5. Dietary exposure assessment to new constituents

In line with Regulation (EU) No 503/2013, the applicant provided dietary exposure estimates to Cry1A.105, Cry2Ab2, Cry1Ac, DMO and CP4 EPSPS proteins newly expressed in soybean MON 87751 × MON 87701 × MON 87708 × MON 89788. Dietary exposure was estimated based on protein expression levels reported in this application for the four-event stack soybean treated with the intended herbicides, the current available consumption data and feed practices, the foods and feeds currently available in the market and the described processing conditions.

Table 7 describes the protein expression levels used to estimate both human and animal dietary exposure.

Table 7: Mean values (n = 20, μg/g dry weight and μg/g fresh weight) for newly expressed proteins in seeds and forage from soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 treated with a combination of the intended herbicides[(a)]

Protein	Tissue/developmental stage	
	Seeds/R8 (μg/g dry weight[(b)] and μg/g fresh weight)	Forage/R6 (μg/g dry weight[(b)])
Cry1A.105	3.7/3.4	360
Cry2Ab2	2.7/2.5	5.6
Cry1Ac	7.7/7.0	100
DMO	13/12.0	18
CP4 EPSPS	100/92	270

(a): Intended herbicides: dicamba and glyphosate.
(b): Dry weight values used to estimate animal dietary exposure were calculated by dividing the values on a fresh weight basis by the dry weight conversion factor obtained from moisture analysis data.

3.6.5.1. Human dietary exposure[25]

Human dietary exposure was estimated across different European countries on different population groups: young population (infants, toddlers, 'other children'), adolescents, adult population (adults, elderly and very elderly) and special populations (pregnant and lactating women).

For the purpose of estimating dietary exposure, the levels of newly expressed proteins in MON 87751 × MON 87701 × MON 87708 × MON 89788 soybean were derived from replicated field trials in the 2013 US growing season (five locations). Mean values (fresh weight basis) are considered as the most adequate to estimate human dietary exposure (see Table 7). Since no specific consumption data were available on commodities containing, consisting of or obtained from MON 87751 × MON 87701 × MON 87708 × MON 89788 soybean, a conservative scenario with 100% replacement of conventional soybean by the GM soybean was considered. Consumption figures for the relevant commodities (soya bean flour, soya bread, textured soy protein, soya drink, soya-based infant formula, soya-based follow-on formula, tofu, etc.) were retrieved from the EFSA Comprehensive European Food

[25] Dossier: Part II – Section 2.4.

Consumption Database (EFSA consumption database).[26] Soybean oil was excluded from the assessment since no proteins are expected to be present in the oil.

For the acute dietary exposure estimations, the applicant estimated the relative amount of each of the newly expressed proteins per gram of soybean protein and multiplied this value by the amount of soybean protein consumed from soybean processed foods. The protein content of the relevant processed foods was derived from the USDA National Nutrient Database,[27] and the consumption data were retrieved from the summary statistics of the EFSA consumption database.[28] This is a conservative approach as neither recipes nor the effect of processing on the final concentration of newly expressed proteins are considered. Acute dietary exposure in high consumers within each dietary survey and age class was estimated by using the food commodity with the highest acute consumption among consumers only (95th or 97.5th percentile depending on the number of consumers). Table 8 shows the highest acute dietary exposure for the different newly expressed proteins; dietary exposure estimates were highest for CP4 EPSPS protein with 270.4 μg/kg body weight (bw) per day and 784.9 μg/kg bw per day in 'other children' and adults, respectively. Most relevant food commodities in terms of contribution to the exposure were soya drink and meat imitates (textured soy protein).

Table 8: Highest acute dietary exposure to Cry1A.105, Cry2Ab2, Cry1Ac, DMO and CP4 EPSPS proteins (μg/kg bw per day) estimated across European dietary surveys and different age classes

	Acute dietary exposure (μg/kg bw per day)				
	Cry1A.105	**Cry2Ab2**	**Cry1Ac**	**DMO**	**CP4 EPSPS**
Other children	10.0	7.3	20.6	35.3	270.4
Adults	29.0	21.3	59.7	102.4	784.9

bw: body weight.

The GMO Panel estimated chronic dietary exposure to Cry1A.105, Cry2Ab2, Cry1Ac, DMO and CP4 EPSPS proteins. Individual consumption data of the relevant food commodities were retrieved from the EFSA Consumption Database, using dietary surveys with at least 2 days consumption and covering a total of 22 European countries.[29] Different recipes and factors were considered to estimate the amount of soybean in the consumed commodities before assigning Cry1A.105, Cry2Ab2, Cry1Ac, DMO and CP4 EPSPS protein levels to the relevant commodities.[30] No losses in the newly expressed proteins during processing were considered. The 95th percentile chronic exposure (highly exposed population) was derived from the distribution of the individual dietary exposure estimates within each dietary survey and age class.

Table 9 shows the highest chronic dietary exposure to each of the newly expressed proteins across European dietary surveys; highest dietary exposure ranged between 0.01 μg/kg bw per day for Cry1A.105 and Cry2Ab2 proteins in infants (< 1 year old) and 73.1 μg/kg bw per day for CP4 EPSPS protein in adolescents (≥ 10 years to < 18 years old). Main average contributors to the exposure in the dietary surveys with the highest estimates were soybean flour in adolescents, and soya drink and soya yoghurt in toddlers. In a scenario where 'consumers only' are considered, the highest dietary exposure estimates in high consumers were 524.4 μg/kg bw per day and 48.1 μg/kg bw per day, for CP4 EPSPS and DMO, respectively.

[26] http://www.efsa.europa.eu/en/data/food-consumption-data
[27] USDA, 2013. U.S. Department of Agriculture, Agricultural Research Service. 2013. USDA National Nutrient Database for Standard Reference, Release 25. Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/ba/bhnrc/ndl.
[28] Summary statistics from the EFSA Comprehensive European Food Consumption Database accessed in July 2015.
[29] Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Germany, Denmark, Estonia, Finland, France, United Kingdom, Greece, Croatia, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Spain, Romania and Sweden.
[30] Example: 100 g of tofu are made with approximately 26 g of soybeans; this would result in 23.9 μg of CP4 EPSPS per gram of tofu as compared to 92 μg/g in the soybeans.

Table 9: Highest chronic dietary exposure estimates (95th percentile, highly exposed population) to Cry1A.105, Cry2Ab2, Cry1Ac, DMO and CP4 EPSPS proteins (μg/kg bw per day) across European dietary surveys and different age classes

	N	Chronic dietary exposure (μg/kg bw per day)				
		Cry1A.105	Cry2Ab2	Cry1Ac	DMO	CP4 EPSPS
Infants	11	0.01	0.01	0.02	0.03	0.2
Toddlers	14	1.7	1.3	3.5	6.0	46.2
Other children	19	1.2	0.9	2.4	4.1	31.4
Adolescents	18	2.7	2.0	5.6	9.5	73.1
Adults	19	0.4	0.3	0.9	1.6	12.1
Elderly	18	0.6	0.4	1.2	2.0	15.3
Very elderly	14	0.1	0.1	0.2	0.4	3.0
Pregnant women	2	0.4	0.3	0.9	1.5	11.4
Lactating women	2	0.1	0.1	0.2	0.4	3.2

bw: body weight; N: number of dietary surveys.

An ad hoc dietary exposure scenario was carried out considering the consumption of protein-based supplements ('Protein and amino acids supplements' and 'Protein and protein components for sports people'), under the assumption that these supplements are prepared from soybean MON 87751 × MON 87701 × MON 87708 × MON 89788. Consumption data on protein-based supplements were available for a total of 14 European countries.[2] The highest average acute dietary exposures (consuming days only) ranged between 27.2 μg/kg bw per day for Cry2Ab2 and 1002.0 μg/kg bw per day for CP4 EPSPS in adults. For high consumers (95th percentile exposure), the highest estimated acute exposures ranged between 40.6 μg/kg bw per day for Cry2Ab2 and 1,495.4 μg/kg bw per day for CP4 EPSPS, also in adults. Similarly, for chronic dietary exposure (consumers only), the highest average estimates ranged between 17.3 μg/kg bw per day for Cry2Ab2 and 637.6 μg/kg bw per day for CP4 EPSPS in adults. Only in one dietary survey, among those reporting consumption of protein-based supplements, was the number of consumers higher than 60 to allow deriving a statistically robust 95th percentile exposure representative of high consumers. The estimated exposure ranged between 0.9 μg/kg bw per day for Cry2Ab2 and 31.5 μg/kg bw per day for CP4 EPSPS in 'other children'.

Furthermore, the consumption data on 'Pollen supplements' reported in the EFSA Consumption Database[27] indicates that additional dietary exposure to the newly expressed proteins can occur under the assumption that these supplements contain pollen from soybean MON 87751 × MON 87701 × MON 87708 × MON 89788. Since no data on the presence of newly expressed proteins in pollen were available, dietary exposure from this source was not estimated.

3.6.5.2. Animal dietary exposure[25]

Animal dietary exposure to Cry1A.105, Cry2Ab2, Cry1Ac, DMO and CP4 EPSPS proteins was estimated following the consumption of soybean meal and soybean forage/silage since these are the two soybean products entering the feed chain. A conservative scenario with 100% replacement of conventional soybean products by the GM products was considered.

Mean levels of Cry1A.105, Cry2Ab2, Cry1Ac, DMO and CP4 EPSPS proteins in soybean seeds and forage/silage were derived from replicated field trial sites (five locations) in the 2013 US growing season (Table 4). To estimate the mean NEP levels in soybean meal, a factor of 1.28-fold was applied based on the protein content of soybean meal relative to soybean seed (OECD, 2012), assuming that no losses of NEP occur during processing.

Dietary exposure to Cry1A.105, Cry2Ab2, Cry1Ac, DMO and CP4 EPSPS proteins in soybean MON87751 × MON87701 × MON87708 × MON89788 following the consumption of soybean meal was provided by the applicant across different animal species (i.e. broiler, finishing swine and dairy cattle), based on estimates for animal body weight, daily feed intake and inclusion rates (percentage) of soybean meal in animal diets (OECD, 2009). Estimated dietary exposure in livestock is reported in Table 10.

Table 10: Dietary exposure to Cry1A.105, Cry2Ab2, Cry1Ac, DMO and CP4 EPSPS proteins (μg/kg bw per day) in livestock

	Dietary exposure (μg/kg bw per day)				
	Cry1A.105	**Cry2Ab2**	**Cry1Ac**	**DMO**	**CP4 EPSPS**
Broiler	133.7	97.5	278.2	469.8	3,614
Finishing swine	42.6	31.1	88.7	149.7	1,152
Dairy cattle	45.5	33.2	94.7	160	1,231

The GMO Panel estimated dietary exposure to Cry1A.105, Cry2Ab2, Cry1Ac, DMO and CP4 EPSPS proteins in dairy cattle following the consumption of soybean forage/silage, based on estimates for animal body weight and daily feed intake (OECD, 2009), and for inclusion rates of soybean forage/silage in animal diets (OECD, 2012). Estimated dietary exposure in dairy cattle is reported in Table 11.

Table 11: Dietary exposure to Cry1A.105, Cry2Ab2, Cry1Ac, DMO and CP4 EPSPS proteins (μg/kg bw per day) in livestock

	Dietary exposure (μg/kg bw per day)				
	Cry1A.105	**Cry2Ab2**	**Cry1Ac**	**DMO**	**CP4 EPSPS**
Dairy cattle	2,770	43	769	138	2,077

3.6.6. Nutritional assessment of endogenous constituents

The intended traits of soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 are herbicide tolerance and insect resistance, with no intention to alter nutritional parameters. None of the compositional differences identified between soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 and the non-GM comparator (see Section 3.5.6) needs further nutritional assessment.

3.6.7. Conclusion of the food and feed safety assessment

The newly expressed proteins Cry1A.105, Cry2Ab, Cry1Ac, DMO and CP4 EPSPS in the four-event stack soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 do not raise safety concerns for human and animal health. No interactions between these proteins relevant for food and feed safety were identified. Similarly, the GMO Panel did not identify indications of safety concerns regarding allergenicity or adjuvanticity related to the presence of the newly expressed proteins in soybean MON 87751 × MON 87701 × MON 87708 × MON 89788, or regarding the overall allergenicity of this four-event stack soybean. Based on the outcome of the comparative assessment, the GMO Panel concludes that the nutritional impact of soybean MON 87751 × MON 87701 × MON 87708 × MON 89788-derived food and feed is expected to be the same as those derived from the comparator and non-GM commercial reference varieties. The GMO Panel concludes that four-event stack soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 as described in this application, is nutritionally equivalent to and as safe as the comparator and the non-GM reference varieties tested.

3.7. Environmental risk assessment and monitoring plan[31]

Considering the scope of the application EFSA-GMO-NL-2016-128, which excludes cultivation, the environmental risk assessment (ERA) of soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 mainly takes into account: (1) the exposure of microorganisms to recombinant DNA in the gastrointestinal tract of animals fed GM material and of microorganisms present in environments exposed to faecal material of these animals (manure and faeces); and (2) the accidental release into the environment of viable soybean MON 87751×MON 87701×MON 87708×MON 89788 seeds during transportation and/or processing (EFSA GMO Panel, 2010a).

[31] Dossier: Part II – Sections 5 and 6.

3.7.1. Persistence and invasiveness of the GM plant

Cultivated soybean (*Glycine max* (L.) Merr.) is a species in the subgenus *Soja* of the genus *Glycine*. The species originated from eastern Asia and is a highly domesticated crop, generally unable to survive in the environment without appropriate 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 and cold climatic conditions (OECD, 2000). Soybean can grow as volunteers and the presence of volunteers of *G. max* was occasionally reported in some areas of Italy where soybean is intensively cultivated (Celesti-Grapow et al., 2010). However, as for the same reasons mentioned above, soybean seeds usually do not survive during the winter (Owen, 2005).

Thus, the establishment and survival of feral and volunteer soybean in the EU is currently limited and transient.

It is unlikely that the intended traits of soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 will provide a selective advantage to soybean plants, except when they are exposed to dicamba- and/or glyphosate-containing herbicides or infested by insect pests that are susceptible to the Cry1A.105, Cry2Ab2 and/or Cry1Ac proteins. However, this fitness advantage will not allow the GM plant to overcome other biological and abiotic factors (described above). Therefore, the presence of the intended traits will not affect the persistence and invasiveness of the GM plant.

In conclusion, the GMO Panel considers it very unlikely that soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 will differ from conventional soybean hybrid varieties in its ability to survive until subsequent seasons, or to establish occasional feral plants under European environmental conditions in case of accidental release into the environment of viable soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 seeds.

3.7.2. Potential for gene transfer

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

3.7.2.1. Plant-to-microorganism gene transfer

The probability and potential adverse effects of HGT of the recombinant DNA have been assessed in previous GMO Panel Scientific Opinions for the single events (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 concern was identified in regard to 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.

The applicant submitted an updated bioinformatic analysis for each of the single events to assess possibility for HGT by homologous recombination.

The updated bioinformatic analysis of events MON 87751, MON 87708 and MON 89788 does not reveal any new DNA sequence that could provide sufficient length and identity which could facilitate HGT by double homologous recombination, confirming the conclusions of the previous GMO Panel Scientific Opinions (EFSA GMO Panel, 2018a, 2019a).

The updated bioinformatic analysis for event MON 87701 reveals one DNA sequence at the left border with sufficient length and identity with bacterial genes from the *A. tumefaciens* Ti plasmid. However, there is no indication for facilitated HGT from MON 87701 to bacteria by double homologous recombination.

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, are not identified.

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

3.7.2.2. Plant-to-plant gene transfer

The potential for occasional feral soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 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 MON 87751 × MON 87701 × MON 87708 × MON 89788 seeds are processed in the countries of production or in ports of importation.

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

Soybean is an annual, almost completely self-pollinating crop with a percentage of cross-pollination usually below 1% (OECD, 2000; Ray et al., 2003; Lu, 2005; Yoshimura et al., 2006; Abud et al., 2007), although natural cross-pollination rates can fluctuate significantly among different soybean varieties under particular environmental conditions, such as favourable climate for pollination and an abundance of pollinators (Caviness, 1966; Gumisiriza and Rubaihayo, 1978; Kikuchi et al., 1993; Ahrent and Caviness, 1994; Ray et al., 2003; Lu, 2005).

The potential of spilled soybean seeds to establish, grow and produce pollen is extremely low and transient (see Section 3.7.1). Therefore, the likelihood/frequency of cross-pollination between occasional feral GM soybean plants resulting from seed spillage, and weedy or cultivated soybean plants is also considered extremely low. Even if cross-pollination would occur, the GMO Panel is of the opinion that the likelihood of environmental effects as a consequence of the spread of genes from occasional feral GM soybean plants in Europe will not differ from that of conventional soybean varieties for the reasons given in Section 3.7.1, even after exposure to dicamba- and/or glyphosate-containing herbicides or infestation by insect pests that are susceptible to the Cry1A.105, Cry2Ab2 and/or Cry1Ac proteins.

3.7.3. Interactions of the GM plant with target organisms

Taking the scope of application EFSA-GMO-NL-2016-128 into account (no cultivation), potential interactions of occasional feral soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 plants arising from seed import spills with the target organism are not considered a relevant issue.

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

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

3.7.5. Interactions with the abiotic environment and biogeochemical cycles

Given that environmental exposure to spilled seeds or occasional feral soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 plants arising from seed import spills is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM soybean, potential interactions with the abiotic environment and biogeochemical cycles are not considered by the GMO Panel to raise any environmental safety concern.

3.7.6. Conclusion on the environmental risk assessment

The GMO Panel concludes that it is unlikely that soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 would differ from conventional soybean varieties in its ability to persist under European environmental conditions. Considering the scope of application EFSA-GMO-NL-2016-128, interactions of occasional feral soybean MON 87751 × MON 87701 × MON 87708 × MON 89788

plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 to bacteria does not indicate a safety concern. Therefore, considering the combined traits and their interactions, the outcome of the comparative analysis and the routes and levels of exposure, the GMO Panel concludes that soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 would not raise safety concerns in the event of accidental release of viable GM soybean seeds into the environment.

3.8. Post-market monitoring

3.8.1. Post-market monitoring of GM food/feed

The GMO Panel concluded that soybean MON 87751 × MON 87701 × MON 87708 × MON 89788, as described in this application, is nutritionally equivalent to and as safe as the non-GM comparator and the non-GM soybean reference varieties tested (Section 3.6.7). Furthermore, the overall intake or exposure is not expected to change because of the introduction of the four-event stack soybean into the market. Therefore, no post-market monitoring (EFSA GMO Panel, 2011a) of food/feed from the four-event stack soybean is considered necessary.

3.8.2. Post-market environmental monitoring

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

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

As the ERA did not identify potential adverse environmental effects from soybean MON 87751 × MON 87701 × MON 87708 × MON 89788, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 includes: (1) the description of a monitoring approach involving operators (federations involved in import and processing), reporting to the applicant, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment; (2) a coordinating system established by EuropaBio for the collection of information recorded by the various operators; and (3) the review of relevant scientific publications retrieved from literature searches (Lecoq et al., 2007; Windels et al., 2008). The applicant proposes to submit a PMEM report on an annual basis and a final report at the end of the authorisation period.

The GMO Panel considers that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of soybean MON 87751 × MON 87701 × MON 87708 × MON 89788. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

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

3.8.3. Conclusion 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 soybean MON 87751 × MON 87701 × MON 87708 × MON 89788.

4. Overall conclusions

The GMO Panel was asked to carry out a scientific assessment of soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003.

No new information on the single soybean events MON 87751, MON 87701, MON 87708 and MON 89788 that would lead to a modification of the original conclusions on their safety were identified.

The molecular characterisation, the comparative analysis (agronomic, phenotypic and compositional characteristics) and the outcome of the toxicological and allergenicity assessments indicate that the combination of the single soybean events and of the newly expressed proteins in the four-event stack

soybean does not give rise to food/feed safety and nutritional concerns. The GMO Panel concludes that the four-event stack soybean, as described in this application, is as safe as and nutritionally equivalent to the non-GM comparator and the non-GM reference varieties tested.

The GMO Panel concludes that there is a very low likelihood of environmental effects resulting from the accidental release of viable seeds from the four-event stack soybean into the environment.

The literature searches did not identify any relevant publications on the four-event stack soybean. In the context of annual PMEM reports, the applicant could further improve future literature searches according to the GMO Panel recommendations.

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

Given the absence of safety and nutritional concerns for foods and feeds from the four-event stack soybean, 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 four-event stack soybean.

In conclusion, the GMO Panel considers that soybean MON 87751 × MON 87701 × MON 87708 × MON 89788, as described in this application, is as safe as the non-GM comparator and the tested non-GM reference varieties with respect to potential effects on human and animal health and the environment.

Documentation as provided to EFSA

- Letter from the Competent Authority of Netherlands received on 23 December 2015 concerning a request for authorisation of the placing on the market of soybean MON 87701 × MON 87708 × MON 87751 × MON 89788 (EFSA-GMO-NL-2016-128) submitted in accordance with Regulation (EC) No 1829/2003 by Monsanto Europe S.A./N.V.
- Application EFSA-GMO-NL-2016-128 validated by EFSA, 22 August 2016.
- Application stopped due to single event, 23 August 2016.
- Request for supplementary information to the applicant, 04 April 2017.
- Receipt of supplementary information from the applicant, 6 June 2017.
- Receipt of supplementary information from the applicant, 19 June 2017.
- Application restarted following adoption of single event, 20 June 2018.
- Request for supplementary information to the applicant, 20 June 2018.
- Request for supplementary information to the applicant, 18 July 2018.
- Receipt of supplementary information from the applicant, 20 July 2018.
- Receipt of supplementary information from the applicant, 13 August 2018.
- Request for supplementary information to the applicant, 21 September 2018.
- Receipt of supplementary information from the applicant, 03 October 2018.
- Receipt of supplementary information from the applicant, 03 October 2018.
- Request for supplementary information to the applicant, 11 October 2018.
- Receipt of supplementary information from the applicant, 14 November 2018.
- Request for supplementary information to the applicant, 12 December 2018.
- Receipt of supplementary information from the applicant, 29 January 2019.
- Request for supplementary information to the applicant, 5 February 2019.
- Receipt of supplementary information from the applicant, 25 February 2019.
- Request for supplementary information to the applicant, 11 April 2019.
- Receipt of supplementary information from the applicant, 06 May 2019.
- Receipt of supplementary information from the applicant, 08 May 2019.
- Receipt of supplementary information from the applicant, 17 May 2019.
- Receipt of supplementary information from the applicant, 4 June 2019.
- Request for supplementary information to the applicant, 14 June 2019.
- Receipt of supplementary information from the applicant, 19 June 2019.
- Receipt of supplementary information from the applicant, 7 August 2019.
- Receipt of spontaneous information from the applicant, 19 August 2019.

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Abbreviations

ELISA	enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate 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
IgE	immunoglobulin E
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PCR	polymerase chain reaction
PMEM	post-market environmental monitoring
UTR	untranslated region

Appendix A – Protein expression data

Mean, standard deviation and range of protein levels (μg/g dry weight) from soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 (treated with dicamba and glyphosate), MON 87751 (not treated), MON 87701 (not treated), MON 87708 (treated with dicamba), MON 89788 (treated with glyphosate) from a field trial performed across five locations in USA in 2013 (n = 20).

Protein	Event(s)	Leaf (V3-V4)	Leaf (V4-V7)	Leaf (R2-R3)	Leaf (R6)	Root (R6)	Forage (R6)	Seed (R8)
Cry1A.105	**MON 87751 × MON 87701 × MON 87708 × MON 89788**	480[(a)] ± 190[(b)] (140–830)[(c)]	250 ± 190 (72–650)	630 ± 320 (230–1,300)	1,400 ± 490 (730–2,600)	< LOQ ± N/A	360 ± 170 (110–790)	3.7 ± 0.94 (2.3–5.7)
	MON 87751	300 ± 160 (51–660)	280 ± 150 (73–690)	420 ± 180 (110–830)	1,800 ± 840 (870–3,700)	< LOQ ± N/A	400 ± 170 (83–780)	4.0 ± 1.3 (2.3–7.4)
Cry2Ab2	**MON 87751 × MON 87701 × MON 87708 × MON 89788**	9.8 ± 4.7 (2.5–17)	19 ± 6.4 7.6–30)	22 ± 5.0 (12–31)	13 ± 2.3 (8.8–18)	7.7 ± 2.9 (4.4–17)	5.6 ± 1.0 (3.4–7.5)	2.7 ± 1.1 (1.2–5.0)
	MON 87751	12 ± 5.0 (4.7–21)	18 ± 6.2 (8.4–29)	17 ± 3.9 (9.4–22)	13 ± 5.1 (8.0–23)	6.6 ± 2.2 (3.9–12)	5.4 ± 1.4 (3.3–8.2)	2.2 ± 0.85 (1.3–4.7)
Cry1Ac	**MON 87751 × MON 87701 × MON 87708 × MON 89788**	300 ± 120 (88–500)	250 ± 110 (100–510)	570 ± 140 (360–800)	2,100 ± 960 (920–4,800)	< LOQ ± N/A	100 ± 37 (52–190)	7.7 ± 1.5 (5.9–12)
	MON 87701	240 ± 98 (91–390)	510 ± 210 (230–950)	560 ± 160 (300–820)	1,800 ± 500 (1,100–3,000)	< LOQ ± N/A	210 ± 160 (94–700)	7.0 ± 1.1 (5.1–9.9)
DMO	**MON 87751 × MON 87701 × MON 87708 × MON 89788**	21 ± 13 (6.3–48)	12 ± 7.1 (4.1–30)	15 ± 4.8 (7.1–26)	23 ± 21 (8.3–110)	4.0 ± 3.7 (0.72–16)	18 ± 4.0 (13–28)	13 ± 3.8 (7.7–20)
	MON 87708	14 ± 7.6 (5.4–30)	12 ± 6.3 (3.3–25)	26 ± 14 (11–69)	30 ± 13 (17–78)	1.3 ± 0.47 (0.44–2.2)	19 ± 7.2 (11–37)	30 ± 5.5 (23–43)
CP4 EPSPS	**MON 87751 × MON 87701 × MON 87708 × MON 89788**	310 ± 190 (130–850)	370 ± 97 (230–580)	460 ± 120 (250–700)	320 ± 65 (220–500)	22 ± 11 (7.2–42)	270 ± 120 (110–510)	100 ± 35 (54–190)
	MON 89788	270 ± 150 (71–600)	240 ± 83 (140–430)	430 ± 120 (270–720)	260 ± 97 (170–440)	64 ± 30 (30–130)	220 ± 80 (110–380)	180 ± 26 (140–230)

DMO: dicamba mono-oxygenase; EPSPS: 5-enolpyruvylshikimate-3-phosphate synthase.
(a): Mean.
(b): Standard deviation.
(c): Range.

Appendix B – List of additional unpublished studies performed by or on behalf of the applicant with regard to the evaluation of the safety of the food and feed for humans, animal and the environment for soybean MON 87751 × MON 87701 × MON 87708 × MON 89788

Study identification	Title
CRO-2013-0142	An Acute Toxicity Study of E. coli-produced Cry1A.105 protein by Oral Gavage in Mice
MSL0022885	Immunodetection of Cry1Ac Following Heat Treatment
MSL0023031	The Effect of Heat Treatment on Dicamba Mono-Oxygenase (DMO) Enzyme Immunodetection
MSL0023754	Amended Report for MSL0022565: Effect of Temperature Treatment on the Functional Activity of Cry1Ac
MSL0026197	An Acute Toxicity Study of E. coli-produced MON 87708 DMO protein by Oral Gavage in Mice
MSL0026236	Amended report for MSL0026192: summary of acute toxicity studies of E. coli-produced Cry2Ab2 protein by oral gavage in mice
MSL0026332	Southern blot analyses to confirm the presence of MON 87751, MON 87701, MON 87708 and MON 89788 in the combined trait soybean product MON 87751 × MON 87701 × MON 87708 × MON 89788
MSL0026454	An acute toxicity study of E. coli-produced CP4 EPSPS protein by oral gavage in mice
MSL0026673	Comparison of broiler performance and carcass parameters when fed diets containing MON 87751 × MON 87701 × MON 87708 × MON 89788, control or reference soybean meal
MSL0026776	An acute oral gavage toxicity study of E. coli-produced MON 87708 DMO protein in CD-1 mice
MSL0026884	Amended Report for MSL0026036: Phenotypic Evaluation of Soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 with Herbicide Treatments in 2013 U.S. Field Trials
MSL0026885	Amended report for MSL0026363: phenotypic evaluation and environmental interactions of soybean MON 87751 × MON 87701 × MON 87708 × MON 89788 in 2013 U.S. field trials
MSL0026935	Amended report for MSL0025737: compositional analyses of soybean seed and forage from MON 87751 × MON 87701 × MON 87708 × MON 89788 grown in the United States in 2013
MSL0026971	Comparison of Gly m 4 expression levels from MON 87751 × MON 87701 ×MON 89788, MON 87751 × MON 87701 × MON 87708 × MON 89788 and conventional soybeans
MSL0027230	Assessment of Cry1A.105, Cry2Ab2, Cry1Ac, DMO and CP4 EPSPS Protein Levels in Soybean Tissues Collected from MON 87751 × MON 87701 × MON 87708 × MON 89788, MON 87751, MON 87701, MON 87708 and MON 89788 Produced in Brazilian Field Trials During 2014/2015 growing season
SCR-2014-0210	Compositional analyses of soybean seed and forage from MON 87751 × MON 87701 × MON 87708 × MON 89788 grown in the United States in 2013