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Assessment of genetically modified soybean MON 87708 × MON 89788 × A5547-127, for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2016-135)

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Abstract

Soybean MON 87708 × MON 89788 × A5547-127 (three-event stack soybean) was produced by conventional crossing to combine three single events: MON 87708, MON 89788 and A5547-127. The GMO Panel previously assessed the three single events and did not identify safety concerns. No new data on the single events, leading to modification of the original conclusions on their safety have been identified. The molecular characterisation, comparative analysis (agronomic, phenotypic and compositional characteristics) and the outcome of the toxicological, allergenicity and nutritional assessment indicate that the combination of the single soybean events and of the newly expressed proteins in the three-event stack soybean does not give rise to food and feed safety and nutritional concerns. The GMO Panel concludes that the three-event stack soybean, as described in this application, is as safe as and nutritionally equivalent to its conventional counterpart and the non-GM reference varieties tested. The nutritional impact of food/feed derived from the three-event stack soybean is expected to be the same as that of food/feed derived from the conventional counterpart and non-GM reference varieties. In the case of accidental release of viable seeds of the three-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 three-event stack soybean. Post-market monitoring of food/feed is not considered necessary. The GMO Panel concludes that the three-event stack soybean is as safe as its conventional counterpart and the tested non-GM reference varieties with respect to potential effects on human and animal health and the environment.

Keywords: GMO, soybean (*Glycine max*), MON 87708 × MON 89788 × A5547-127, Regulation (EC) 1829/2003, DMO, CP4 EPSPS, PAT, import and processing

Requestor: Competent Authority of Netherlands

Question number: EFSA-Q-2016-00688

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Summary

Following the submission of application EFSA-GMO-NL-2016-135 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 87708 × MON 89788 × A5547-127 (hereafter referred to as 'the three-event stack soybean'). The scope of application EFSA-GMO-NL-2016-135 is for the placing on the market of the three-event stack soybean for food and feed uses, import and processing.

The three-event stack soybean was produced by conventional crossing to combine three single soybean events: MON 87708 (producing DMO), MON 89788 (producing CP4 EPSPS) and A5547-127 (producing PAT), to confer tolerance to dicamba, glyphosate and glufosinate-ammonium containing herbicides.

The GMO Panel evaluated the three-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 three-event stack soybean, the scientific comments submitted by the Member States and the relevant scientific literature.

The single events MON 87708, MON 89788 and A5547-127 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 three 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 three-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 three-event stack soybean have retained their integrity. Protein expression analyses show that the levels of the newly expressed proteins are comparable in the three-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 three-event stack soybean are identified.

The comparative analysis of forage and seed composition and agronomic/phenotypic characteristics identified no differences between the three-event stack soybean and its conventional counterpart that required further assessment for food/feed safety or environmental impact, except for the levels of acid detergent fibre, total fat and behenic acid in seeds. All 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, allergenicity and nutritional assessment indicate that the combination of the single soybean events and of the newly expressed proteins in the three-event stack soybean does not give rise to food and feed safety and nutritional concerns. The GMO Panel concludes that soybean MON 87708 × MON 89788 × A5547-127, as described in this application, is as safe as and nutritionally equivalent to its conventional counterpart 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 87708 × MON 89788 × A5547-127 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 three-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 three-event stack soybean. The literature searches did not identify any relevant publications on the three-event stack soybean. In the context of annual PMEM reports, the applicant should improve future literature searches according to the GMO Panel recommendations.

The GMO Panel concludes that soybean MON 87708 × MON 89788 × A5547-127, as described in this application, is as safe as its conventional counterpart 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-2016-135 is for food and feed uses, import and processing in the European Union (EU) of the genetically modified (GM) herbicide tolerant soybean MON 87708 × MON 89788 × A5547-127.

1.1. Background

On 3 November 2016, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands the application EFSA-GMO-NL-2016-135 for authorisation of soybean MON 87708 × MON 89788 × A5547-127 (Unique Identifiers MON-877Ø8-9 × MON-89788-1 × ACS-GMØØ6-4), submitted by Monsanto (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003.[1]

Following receipt of application EFSA-GMO-NL-2016-135, EFSA informed the EU Member States (MS) 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 19 January 2017, EFSA declared the application valid, and made the valid application available to the EU MS and EC.

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 6 months to issue a scientific opinion on application EFSA-GMO-NL-2016-135. 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 MS 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 MS, including national Competent Authorities within the meaning of Directive 2001/18/EC.[4] The EU MS had 3 months to make their opinion known on the application EFSA-GMO-NL-2016-135 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 87708 × MON 89788 × A5547-127 in the context of its scope as defined in application EFSA-GMO-NL-2016-135.

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 MS.[5]

In addition to the present scientific opinion on soybean MON 87708 × MON 89788 × A5547-127, 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 information required under Annex II to the Cartagena Protocol, a labelling proposal as well as a Post-Market Environmental Monitoring (PMEM) plan as provided by the applicant are made available in the EFSA Register of Questions.[6] Whereas the method (s), validated by the Community Reference Laboratory, for detection, including sampling, identification of the transformation event in the food-feed and/or foods-feeds produced from it can be found at http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx, [Validation report MON 87708 × MON 89788 ×

[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/questionDocumentsLoader?question=EFSA-Q-2016-00688).

[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 MS can be found at the EFSA Register of Questions (http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2016-00688).

[6] http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2016-00688

A5547-127; Validated methods: MON 87708, MON 89788, A5547-127; DNA extraction]. The appropriate reference materials can be accessed at https://www.aocs.org/ (MON 87708, MON 89788, A5547-127).

2. Data and methodologies

2.1. Data

The GMO Panel based its scientific risk assessment of soybean MON 87708 × MON 89788 × A5547-127 on the valid application EFSA-GMO-NL-2016-135, additional information provided by the applicant during the risk assessment, relevant scientific comments submitted by EU MS and relevant peer-reviewed scientific publications. In addition to this comprehensive information package, the GMO Panel also received unpublished studies submitted by the applicant in order to comply with the specific 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 (i.e., EFSA GMO Panel, 2010a,b, 2011a,b, 2015a) and explanatory notes (i.e., EFSA, 2014, 2017a,b) 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 2011 EFSA Scientific Committee guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed 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).

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-2016-135 covers soybean MON 87708 × MON 89788 × A5547-127. This three-event stack was produced by conventional crossing to combine three single soybean events: MON 87708 (expressing dicamba mono-oxygenase (DMO protein)), MON 89788 (expressing the 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS protein)) and A5547-127 (expressing phosphinothricin acetyltransferase (PAT protein)), to confer tolerance to dicamba, glyphosate and glufosinate-ammonium-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, 2015a, 2018).

The three 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 already assessed by the GMO Panel

Events	Application or mandate	Reference
MON 87708	EFSA-GMO-NL-2011-93	EFSA GMO Panel (2013)
MON 89788	EFSA-GMO-NL-2006-36	EFSA (2008)
A5547-127	EFSA-GMO-NL-2008-52	EFSA GMO Panel (2011c)

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 three single events has been reported by the applicant.

Updated bioinformatic analyses for soybean events MON 87708, MON 89788 and A5547-127 confirm that no known endogenous genes were disrupted by any of the inserts.

Updated bioinformatic analyses of the amino acid sequence of the newly expressed DMO, CP4 EPSPS and PAT proteins confirm previous results indicating no significant similarities to toxins and allergens. Updated bioinformatic 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 87708, MON 89788 and A5547-127, confirm previous analyses, indicating that the expression of an ORF showing significant similarities to toxins or allergens is highly unlikely (see Table 1).

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 87708, MON 89788 and A5547-127 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 87708 × MON 89788 × A5547-127, which included a scoping review, according to the guidelines given in EFSA (2010).

A systematic review as referred to in Regulation (EU) No 503/2013 has not been provided in support to the risk assessment of application EFSA-GMO-NL-2016-135. Based on the outcome of the scoping review, the GMO Panel agrees that there is limited value in undertaking a systematic review for soybean MON 87708 × MON 89788 × A5547-127 at present.

Although the overall quality of the performed literature searches is acceptable, the GMO Panel considers that future searches on soybean MON 87708 × MON 89788 × A5547-127 should 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 used consistently;
- where subject headings are available use both free-text terms and controlled vocabulary in the searches;
- adapt the search to the size of the identified publications (and thus not combine search sets when one of the search sets already yields only a small number of publications);
- assess the relevance and risk assessment implications of publications retrieved via searches beyond electronic bibliographic databases.

The literature searches did not identify any relevant publications on soybean MON 87708 × MON 89788 × A5547-127.

3.4. Molecular characterisation

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

3.4.1. Genetic elements and their biological function[10]

Soybean events MON 87708, MON 89788 and A5547-127 were combined by conventional crossing to produce the soybean MON 87708 × MON 89788 × A5547-127. The structure of the inserts

[7] Additional information: 31/5/2018.
[8] Part II Scientific information, Section 1.2.2.2.
[9] Dossier: Part II – Section 7; additional information: 31/5/2018.
[10] Part II Scientific information, Section 1.2.2.2 and additional information: 25/01/2017, 31/5/2018.

introduced into the three-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.

Intended effects of the inserts in soybean MON 87708 × MON 89788 × A5547-127 are summarised in Table 3.

Based on the known biological function of the newly expressed proteins (Table 3), no foreseen interactions at the biological level are expected.

Table 2: Genetic elements in the expression cassettes of the events stacked in soybean MON 87708 × MON 89788 × A5547-127

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
MON 87708	Full-length transcript promoter from *Peanut chlorotic streak* virus	5′ UTR from *Tobacco etch* virus	*RbcS* (*Pisum sativum*)	*dmo* (*Stenotrophomonas maltophilia*)	3′ UTR of *RbcS2* (*Pisum sativum*)
MON 89788	35S promoter from *Figwort mosaic* virus and promoter from the Tsf1 gene of *Arabidopsis thaliana*	5′ UTR and intron from *Tsf1* gene of *Arabidopsis thaliana*	*ShkG* (*Arabidopsis thaliana*)	CP4 *epsps* (*Agrobacterium tumefaciens* strain CP4)	3′ UTR of *RbcS2* (*Pisum sativum*)
A5547-127	35S promoter from *Cauliflower mosaic* virus (CaMV)	–	–	*pat* (*Streptomyces viridochromogenes*)	*t35S* (CaMV)

UTR: untranslated region.
–: when no element was specifically introduced to optimise expression.

Table 3: Characteristics and intended effects of the events stacked in soybean MON 87708 × MON 89788 x A5547-127

Event	Protein	Donor organism and biological function	Intended effects in GM plant
MON 87708	DMO	Based on a gene from *S. 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
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
A5547-127	PAT	Based on a gene from *S. viridochromogenes*, Tü494 phosphinothricin-acetyl-transferase (PAT) enzyme acetylates L-glufosinate-ammonium (Thompson et al., 1987; Wohlleben et al., 1988; Eckes et al., 1989)	Event A5547-127 expresses the PAT protein, which confers tolerance to glufosinate-ammonium-containing herbicides (Droge-Laser et al., 1994)

3.4.2. Integrity of the events in the three-event stack soybean[11]

The genetic stability of the inserted DNA over multiple generations in the single soybean events MON 87708, MON 89788 and A5547-127 was demonstrated previously (see Table 1). Integrity of these genetically independent events in soybean MON 87708 × MON 89788 × A5547-127 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 three-event stack soybean are identical to the sequences originally reported for the three single events.

[11] Part II Scientific information, Section 1.2.2.2 and additional information: 31/5/2018.

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

DMO, CP4 EPSPS and PAT protein levels were analysed by enzyme-linked immunosorbent assay (ELISA) in material harvested from a field trial at five locations in the USA in 2015. Samples analysed included leaf (V3–V4 and R6), seed (R8), root (R6) and forage (R6) treated with the 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 three-event stack soybean and the corresponding single events in different parts of the plant.

The levels of all the newly expressed proteins in the three-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. Conclusion of the molecular characterisation

The molecular data establish that the events stacked in soybean MON 87708 × MON 89788 × A5547-127 have retained their integrity. Protein expression analyses show that the levels of the newly expressed proteins are comparable in the three-event stack and in the single events. Therefore, there is no indication of 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, no foreseen interactions at the biological level are expected.

3.5. Comparative analysis[13]

3.5.1. Overview of studies conducted for the comparative analysis

Application EFSA-GMO-NL-2016-135 presents data on agronomic and phenotypic characteristics, as well as on forage and seed composition of the three-event stack soybean (Table 4).

Table 4: Overview of the comparative analysis studies to characterise the three-event stack soybean provided in application EFSA-GMO-NL-2016-135

Study focus	Study details	Comparator	Non-GM reference varieties
Agronomic and phenotypic analysis	Field study, USA, 2015, eight sites(a)	A3555	16(b)
Compositional analysis			

GM: genetically Modified.

(a): The field trials were located in Boone, IA; Warren, IL; Bureau, IL; Pawnee, KS; Perquimans, NC; York, NE; Miami, OH and Lehigh, PA.

(b): Non-GM reference varieties used in the 2015 field trials, with their corresponding maturity group indicated in brackets were Great Lakes GL3029 (3.0); ILLINI 3477N (3.4); ILLINI 3880B (3.8); Becks 319N (3.1); A3525 (3.5); Stine 3822-2 (3.8); A3253 (3.2): LG C3554 (3.5); Stine 3900-2 (3.9); ILLINI 6336N (3.2); Becks 389N (3.8); WILLIAMS 82 (3.9); Becks 331N (3.3); HiSoy HS 38C60 (3.8); Stine 33E22 (3.3) and Great Lakes GL3809 (3.8).

3.5.2. Experimental field trial design and statistical analysis

At each field trial site, the following materials were grown: the three-event stack soybean, the comparator A3555 and four of the sixteen commercial non-GM soybean reference varieties (hereafter 'non-GM reference varieties'). All materials were treated with conventional herbicide management regimes; in addition the field trials included the three-event stack soybean exposed to the intended dicamba, glyphosate and glufosinate-ammonium containing herbicides on top of the conventional herbicides.

The agronomic/phenotypic and compositional data were analysed as specified by the GMO Panel (2010b, 2011a). This includes, for each of the two treatments of the three-event stack soybean, the application of a difference test (between the GM soybean and the comparator) and an equivalence test (between the GM soybean and the set of non-GM reference varieties).[14] The results of the

[12] Dossier: Part II – Section 1.2.2.3 and additional information: 16/6/2017 and 13/11/2018.

[13] Dossier: Part II – Section 1.3; additional information: 21/4/2017 and 28/8/2017.

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

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 three-event stack GM soybean, events MON 87708 and MON 89788 were combined by conventional crossing in soybean variety A3525. Soybean MON 87708 × MON 89788 was then crossed with soybean variety Benning harbouring event A5547-127 before being backcrossed and stabilised in soybean variety A3555.

The comparator used in the field trials is the non GM soybean variety A3555, which has a genetic background similar to that of soybean MON 87708 × MON 89788 × A5547-127 (as documented by the pedigree and by the additional information), and is therefore considered the conventional counterpart.

Both the three-event stack soybean and its conventional counterpart belong to maturity group 3.5, which is considered appropriate for growing in environments across North America, where the comparative field trials were conducted.

3.5.3.2. Selection of non-GM reference varieties

The 16 non-GM reference varieties with maturity groups ranging from 3.0 to 3.9 were selected by the applicant and at each field trial site four of them were tested (see Table 4). On the basis of the information provided on relative maturity classes, the GMO Panel considers the selected non-GM reference varieties appropriate for the comparative assessment.

3.5.3.3. Seed production and quality

Seeds of the three-event stack soybean and the conventional counterpart were produced, harvested and stored under similar conditions, before being sown in the field trials. The seed lots were verified for their purity via event specific quantitative PCR analysis. The mean germination rates of the three-event stack soybean and its conventional counterpart were 96% and 98% respectively. The GMO Panel considers that the starting seed used as test material in the agronomic, phenotypic and compositional studies was of acceptable quality.

3.5.3.4. Conclusion on suitability

The GMO Panel is of the opinion that the three-event stack soybean, its conventional counterpart and the non-GM reference varieties were properly selected and of adequate 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 dataset falls within the range of climatic conditions normally occurring at these sites.

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

[16] Soil types of the field trials were silty clay loam, loam and silt loam; soil organic matter ranged from 1.9% to 5.3% except for site in Perquimans, NC (41.4%).

3.5.4.3. Management practices

The field trials included plots containing the three-event stack soybean, plots with the conventional counterpart and plots with non-GM reference varieties, all managed according to local agricultural practices. In addition, the field trials included plots containing the three-event stack soybean managed following the same agricultural practices, plus exposed to the intended herbicides. A mix of the intended dicamba and glyphosate-containing herbicides was applied at Biologische Bundesanstalt, Bundessortenamt and Chemical Industry (BBCH) 13–15 growth stage.[17] In addition, a glufosinate-ammonium-containing herbicide was applied at BBCH 15–16 growth stage[18] at least 7 days after the dicamba and glyphosate application. The GMO Panel considers that the management practices including sowing, 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 test materials could be grown.

3.5.5. Agronomic and phenotypic analysis

Nine agronomic and phenotypic endpoints[19] plus abiotic stressors, disease incidence and arthropod damage were collected from the 8 sites (see Table 4). The endpoint pod shattering was not subjected to a formal statistical analysis because more than 90% of the values were 0.

The statistical analysis (Section 3.5.2) was applied to eight endpoints, with the following results:

- For soybean MON 87708 × MON 89788 × A5547-127 treated with conventional herbicides, the test of difference identified statistically significant differences from the conventional counterpart for days to 50% flowering and seed moisture. The values of both endpoints fell under equivalence category I.
- For soybean MON 87708 × MON 89788 × A5547-127 treated with the intended herbicide, the test of difference identified statistically significant differences from the conventional counterpart for early stand count, days to 50% flowering, plant height, seed moisture and seed weight. The values of all the endpoints fell under equivalence category I.

3.5.6. Compositional analysis

Soybean MON 87708 × MON 89788 × A5547-127 seeds and forage harvested from 8 sites (Table 4) were analysed for 74 constituents (seven in forage and 67 in seeds), including those recommended by OECD (2012). Among the constituents analysed, 14 fatty acids (FA) in seeds were excluded from the statistical analysis since more than 50% of the observations were below the limit of quantification.[20]

The statistical analysis was applied to a total of 60 constituents (53 in seeds[21] and 7 in forage[22]); a summary of the outcome of the test of difference and the test of equivalence is presented in Table 5:

[17] BBCH scale describes phenological stages (Meier, 2001) and BBCH 13-15 corresponds to approximately V2–V4 stages of soybean development (Fehr and Caviness, 1977).
[18] BBCH 15–16 corresponds to approximately V4–V5 stages of soybean development.
[19] Early stand count, days to 50% flowering, final stand count, plant height, plant lodging, pod shattering, seed moisture, seed weight, yield.
[20] 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).
[21] Seed constituents included proximates and fibre content (ash, carbohydrates, moisture, protein, total fat, acid detergent fibre (ADF) and neutral detergent fibre (NDF)), minerals (calcium and phosphorus), 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 (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) and behenic acid (22:0)), vitamins (vitamin E (α-tocopherol) and vitamin K1 (phylloquinone)), isoflavones (daidzein, genistein, glycitein), other compounds (phytic acid, raffinose, soybean lectin, stachyose, trypsin inhibitor, gly m 5 (β-conglycinin), gly m 6 (glycinin), gly m Bd 28k, gly m Bd 30k, gly m 1, gly m 3, gly m 4 and gly m 8).
[22] Forage constituents included moisture, protein, total fat, ash, carbohydrates, acid detergent fibre (ADF) and neutral detergent fibre (NDF).

- For soybean MON 87708 × MON 89788 × A5547-127 treated with the intended herbicides, all the 31 seed endpoints for which significant differences were found between the GM soybean and the conventional counterpart fell under equivalence category I or II, except for ADF, total fat and behenic acid, the first two falling under equivalence category III and the behenic acid under equivalence category IV. No statistically significant differences were found for any of the seven forage constituents, and all fell under equivalence category I or II.
- For soybean MON 87708 × MON 89788 × A5547-127 treated with conventional herbicides, all the 25 seed endpoints for which significant differences were found between the GM soybean and the conventional counterpart fell under equivalence category I or II, except for behenic acid that fell under equivalence category IV. No statistically significant differences were found for any of the seven forage constituents, and all fell under equivalence category I or II.

Table 5: Outcome of the comparative compositional analysis in seeds and forage for soybean MON 87708 × MON 89788 × A5547-127. 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	28	28[d]	34	24[d]
	Category III/IV	–	3[e]	–	1[e]
	Not categorised	1[f]	–	1[f]	–
	Total endpoints	60		60	

(a): Comparison between soybean MON 87708 × MON 89788 × A5547-127 and its conventional counterpart.
(b): Four different outcomes: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence). Not categorised means that the test of equivalence was not applied because of the lack of variation among the non-GM reference varieties.
(c): Treated/not-treated with intended herbicides dicamba, glufosinate-ammonium and glyphosate.
(d): Endpoints with significant differences between soybean MON 87708 × MON 89788 × A5547-127 and its conventional counterpart falling in equivalence category I-II (treated and not-treated).
For seeds, both treated and not-treated: arginine, aspartic acid, glutamic acid, glycine, histidine, leucine, lysine, proline, serine, threonine, valine, stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), daidzein, genistein, protein, carbohydrates, vitamin E (α-tocopherol), vitamin K1 (phylloquinone). Only treated: phenylalanine, tyrosine, phytic acid, stachyose, moisture, neutral detergent fibre and gly m 3. Not-treated: trypsin inhibitor, palmitic acid (16:0) and ash.
(e): Endpoints with significant differences between the soybean MON 87708 × MON 89788 × A5547-127 and its conventional counterpart and falling in equivalence category III-IV. Estimated means are reported for these endpoints in Table 7.
(f): Endpoints not categorised for equivalence and without significant differences between the MON 87708 × MON 89788 × A5547-127 and its conventional counterpart: glycinin.

The GMO Panel assessed all significant differences between soybean MON 87708 × MON 89788 × A5547-127 and its conventional counterpart, taking into account the potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties. Mean estimates for the endpoints showing significant differences between soybean MON 87708 × MON 89788 × A5547-127 and its conventional counterpart and falling under category III/IV are given in Table 6.

Table 6: Quantitative results (estimated means and equivalence limits) for endpoints with significant differences between soybean MON 87708 × MON 89788 × A5547-127 and its conventional counterpart and falling under category III and category IV in the test of equivalence (see Table 5)

	Endpoint	Soybean MON 87708 × MON 89788 × A5547-127[(a)]		Conventional counterpart	Non-GM reference varieties	
		Not-treated	Treated		Mean	Equivalence limits
Seeds	Acid detergent fibre (% dw)	13.90	15.63*	14.12	14.33	(13.07–15.59)
	Total fat (% dw)	18.79	18.26*	18.73	20.09	(18.28–21.91)
	Behenic acid (22:0) (%FA)	0.27*	0.27*	0.30	0.33	(0.29–0.36)

GM: genetically modified.

(a): For the soybean MON 87708 × MON 89788 × A5547-127, significantly different values are marked with an asterisk, while the outcomes of the test of equivalence are differentiated by greyscale backgrounds: white (equivalence category I or II), light grey (equivalence category III) and dark grey (equivalence category IV). dw: dry weight; FA: fatty acids. Treated: treated with herbicides dicamba, glufosinate-ammonium and glyphosate; not-treated: treated only with conventional herbicides (see Section 3.5.4.3).

3.5.7. Conclusions on the comparative analysis

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

1) None of the differences identified in the agronomic and phenotypic characteristics tested between the three-event stack soybean and its conventional counterpart needs further assessment regarding their potential environmental impact.
2) None of the differences identified in forage and seed composition between the three-event stack soybean and its conventional counterpart needs further assessment regarding food and feed safety, except for the levels of ADF (treated GM), total fat (treated GM) and behenic acid (treated and not-treated GM) in seeds, which are further assessed in Sections 3.6.3 and 3.6.6.

3.6. Food and feed safety assessment

3.6.1. Effects of processing

Soybean MON 87708 × MON 89788 × A5547-127 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 three-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 CP4 EPSPS, DMO and PAT have been previously evaluated by the GMO Panel (Table 1). No new information has been provided in the context of this application.

3.6.3. Toxicology

3.6.3.1. Testing of newly expressed proteins[23]

Three proteins (CP4 EPSPS, DMO and PAT) are newly expressed in the three-event stack soybean (Section 3.4.1). The GMO Panel has previously assessed these proteins in the context of the single soybean events (Table 1), and no safety concerns were identified for humans and animals. The GMO Panel is not aware of any new information that would change these conclusions.

[23] Dossier: Part II – Section 1.4.1.

The potential for a functional interaction between the proteins newly expressed in soybean MON 87708 × MON 89788 × A5547-127 has been assessed with regard to human and animal health. The CP4 EPSPS, DMO and PAT proteins are enzymes catalysing distinct biochemical reactions and acting on unrelated substrates with high substrate specificity. On the basis of the known biological functions of the individual newly expressed proteins (Table 3), there is currently no expectation for possible interactions relevant to the food and feed safety of the three-event stack soybean.

In vitro protein degradation studies on CP4 EPSPS, DMO and PAT proteins have been previously evaluated by the GMO Panel and no indications of safety concerns were identified (Table 1). In the context of this application, no new studies addressing *in vitro* protein degradation of these newly expressed proteins were provided by the applicant.

The GMO Panel concludes that there are no safety concerns for human and animal health related to the proteins CP4 EPSPS, DMO and PAT newly expressed in the three-event stack soybean.

3.6.3.2. Testing of new constituents other than proteins

No new constituents other than newly expressed proteins have been identified in the three-event stack soybean. 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

Acid detergent fibre, total fat and behenic acid were significantly different in the three-event stack soybean when compared with its conventional counterpart and showed a lack of equivalence with the non-GM reference varieties (Section 3.5.6). Taking into account the biological characteristics and functions of these compounds, the observed differences are considered of no toxicological concern. Further information on the safety of these soybean constituents is provided in Section 3.6.6.

3.6.3.4. Testing of the whole genetically modified food and feed

Based on the outcome of the molecular characterisation assessment, 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 the three-stack soybean have been identified (see Sections 3.4.4, 3.5.6 and 3.6.3). Therefore, animal studies on food/feed derived from the three-event stack soybean 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 soybean event MON 87708, MON 89788 and A5547-127. The three studies had already been provided in the context of the single-event applications and assessed by the GMO Panel; no adverse effects related to the administration of the respective GM diets had been identified (Table 1). In the context of the assessment of this three-event stack soybean 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 histopathological analysis and missing information on test material and diets for the 90-day study on MON 87708; and new studies on MON 89788 and A5547-127, since the previously provided were conducted at low dose.

These studies are adapted from OECD TG 408 (1998) and comply with the principles of Good Laboratory Practice (GLP), except for the lack of analytical determination of concentration, homogeneity and stability of the test item in the formulated diets. It is recognised that it may not always be technically possible to generate information on homogeneity and concentration for a test item administrated or formulated, and the lack of such data and its impact on the validity of a study should be justified (OECD, 2018). The GMO Panel acknowledges that there are no practical methods available to analytically determine these for complex test items such as soybean meal in formulated diets and considers adequate the application of proper diet preparation procedures and regular evaluations of the mixing methods. Based on the additional information received from the applicant, the GMO Panel considers that the diet preparation procedures in place in the facilities where the diets for the three studies were prepared guaranteed their homogeneity and the proper concentration of the respective test or control items. As regards the stability of the test item (soybean meal) in the diets, the applicant considers that in accordance to product expiration standards declared by the diet manufacturer the constituents of the diets used in these studies are stable for the duration of the treatment. The GMO Panel considers this justification acceptable. In addition, the GMO Panel notes that even though the diets were prepared and analysed in non-GLP facilities, standardised procedures

and quality measures were followed. Therefore, the GMO Panel considers that this is not a major deviation impacting these studies.

As regards the 90-day study on MON 87708, the additional histopathology analysis[24] revealed minimal bone marrow (sternum) hypocellularity in 2/12 females given the 30% test diet. This finding was not associated with changes in related endpoints (e.g. haematology) and therefore, not considered as being adverse. Other sporadic histopathological findings observed in this additional histopathology dataset are considered compatible with the spontaneous background pathology of rats of this strain and age. Therefore, the GMO Panel considers that the study is in line with Regulation (EU) 503/2013 and confirms its previous conclusions that no adverse effects were observed in rats given diets formulated with 15% or 30% toasted and defatted meal from soybean MON 87708.

In the new 90-day study on MON 89788,[25] 96 Crl:CD(SD) rats (48 per sex) were randomly allocated to three treatment groups (control, GM-low-dose and GM-high-dose group, n = 16/sex per group) using a stratified complete block design.[26] The groups were fed diets containing 30% or 15% (w/w) defatted toasted meal from MON 89788 soybean sprayed with glyphosate-containing herbicide (test diets) or 30% defatted toasted meal from the conventional counterpart (A5547, control diet).[27] Prior to their processing into defatted toasted meal, the control and MON 89788 seeds were stored at 4°C up to 27 months. The applicant declares that soybean seeds under these storage conditions and with a similar moisture content (12–13%) are described to be stable for up to 36 months, therefore the GMO Panel accepts the use of soybean seeds from MON 89788 and A5547 as starting materials for the study. The applicant declares that the presence of the MON 89788 event was confirmed in seeds before processing to meal. Balanced diets were prepared according to the specifications for PMI Certified Rodent LabDiet#5002. Test and control materials, as well as test and control diets were analysed for proximates, amino acids, minerals, mycotoxins and pesticides (including glyphosate). In-life procedures and observations and terminal procedures were conducted in accordance to OECD TG 408 (1998). Mean, median, standard deviation, min and max were reported for all continuous endpoints for each group and sex and per period or time as appropriate. The statistical analysis was performed using mixed models with treatment, sex and their interaction as fixed effects.[28] For locomotor activity data, a dedicated model was set up.[29] Only when the interaction of the treatment per sex was significant, the comparison of the GM groups (30% and 15%) versus the control group was conducted separately for the two genders. Data were analysed by cage when the cage random effect was significantly different from zero, by animal otherwise. No mortality was observed during the study.

No ophthalmoscopic findings were seen. Increased incidence of red staining around the nose was observed in females given 30% test diet. This finding is considered not adverse since it was observed in a limited number of animals on a small number of occasions (22/1,232 observations, with 15 of the 22 in just 2 animals), it was not associated with changes at functional observation batteries or at macroscopic examination. No statistically significant differences were noted in mean body weight between test diet-given animals and controls. Statistically significant higher mean feed consumption (as g/cage per day and g/kg cage per day) was observed in females given 30% test diet (~20%) in study week interval 2–3 as compared to concurrent controls. The GMO Panel considers this isolated finding not to be treatment related. Statistically significant differences in palpebral closure (*home cage observation*), ease of removal and posture *(handling*) were noted in animals given 15% and 30% test diet as compared to controls. The GMO Panel considers that these findings are not adverse, being a limited segment of the test battery, showing no progression with increased dose and representing relatively small changes. Statistically significantly higher urination was noted at *open field observation* in rats fed the 15% test diet as compared to controls. This finding is considered incidental not showing dose relationship. No statistically significant differences in motor activity were observed between animals given 30% or 15% test diet as compared to concurrent controls. Statistically significant

[24] Aorta, bone (sternum) with bone marrow, cecum, cervix (females only), eyes with optic nerves, lung (including bronchi), mandibular lymph node, Peyer's Patches, skin with mammary gland (females only), skin from males (similar area), oesophagus, pituitary, prostate (males only), mandibular salivary gland, seminal vesicles (males only), skeletal muscle, trachea, urinary bladder, uterus (females only), and vagina (females only) from all animals given the control and 30% test diet.

[25] Additional information 4/7/2018, Study MSL0029552.

[26] For each sex, animals were stratified by body weight and randomly assigned by group of six to eight blocks. Within each block, the animals were randomly assigned to the three treatment groups and housed in pairs.

[27] Additional information 14/8/018.

[28] The random effects included only the block for food consumption, while for the other outcomes the interaction block per treatment was also considered to test for the cage effect.

[29] Time effect and its interaction with the other fixed and random effects as additional factors was considered.

decreased white blood cell (WBC) parameters were noted in rats given the 30% test diet when compared to concurrent controls. These consisted in decreased WBC count (10% in females and 19% in males), neutrophils (8% in females ad 23% in males), lymphocytes (11% in females and 18% in males), basophils and large unstained cells (LUC). The same trend was noted in groups given the 15% test diet when compared to controls, even though no statistical significance was achieved. The actual mean values of these parameters from rats given the test diets fall within the historical control data (HCD) range.[30] The only exception is the mean WBC count in males administered the 30% test diet, just minimally below the lower limit of the range of mean values from HCD; however, individual data are within the range of historical controls. These finding are not associated with histopathological changes in the bone marrow or in other lymphoid organs. The GMO Panel considers these findings not adverse, being the values within the natural variability of rats of this strain, age and in these experimental conditions. Coagulation parameters did not show statistically significant differences between groups given the test diets and controls. At clinical chemistry, statistically significant differences are observed for albumin/globulin ratio, glucose (males only) and sodium (females only) when comparing the 30% test diet and the controls; for potassium, triglycerides and eosinophil count when comparing the 15% test diet and the control. These minimal differences are considered not to be adverse. Statistically significant lower kidney weight (absolute) was noted in animals fed the 30% and 15% test diets compared to the control (around 5% and 7%, respectively). A significant lower weight was also observed in males fed the 30% test diet as for the epididymides (around 6%). These minimal changes are considered not adverse because they are not reflected in changes in the relative-to-body organ weight and not associated with histopathological findings. At necropsy, no gross pathological findings related to the administration of the test diets were seen. At histopathological examination, follicular cysts and/or decreased corpora lutea were noted in the ovaries of one animal given the control diet and in a few females given the 15% (2/16) and 30% (3/16) test diet. These rats also showed thickened epithelium with increased keratin in the vagina. Following an additional peer review of the data, the test facility concluded that these morphological features, i.e. ovarian follicular cysts (non-ovulatory follicles) with subsequent decreased corpora lutea and keratinisation of the vaginal epithelium represent a common finding in Sprague–Dawley rats of the age of those used in the study and is often described as the persistent oestrus phase of reproductive senescence. The GMO Panel noted that the difference in incidence between control and treated groups is not statistically significant.[31] In addition, decreased incidence of proestrus was noted in females given 15% (2/16) and 30% (2/16) test diet as compared to controls (9/16). The historical control data over several periods confirm that the finding of proestrus consistently has a very high variability (from 10% to 100% of animals) and should not be considered as an adverse finding in isolation. No statistically significant differences in ovaries weight or in the uterus weight of test diet fed rats as compared to controls were noted. Based on the available information, the GMO Panel is of the opinion that findings observed in the female genital tract are the manifestation of a normal variability of rats of this strain and age. The GMO Panel concludes that the 90-day study on MON 89788 is in line with the requirements of Regulation (EU) 503/2013 and that no adverse effects were observed in rats after feeding diets including 15% and 30% defatted toasted meal from soybean MON 897988 for 90 days.

In the new 90-day study on A5547-127 soybean, 96 Crl:CD(SD) rats (48 per sex) were randomly allocated to three treatment groups (control, GM and reference group, n = 16/sex per group) using a stratified complete block design.[26] The groups were given diets containing approximately 30% (w/w) defatted toasted meal from A5547-127 sprayed with glufosinate-containing herbicide (test diet), from the conventional counterpart (A5547, control diet) and from a commercial variety (reference diet). The reference group was introduced to provide additional data on the variability of the study endpoints; however, no statistical comparisons were performed between the reference group and the other groups. The seeds used to produce the test and control materials were sent to the processing facility within 24 h from harvest, maintained at 13–19°C for 5 days and then processed into defatted toasted meal. The identity of A5547 meal and diet was confirmed by PCR. Balanced diets were prepared according to the specifications for PMI Certified Rodent LabDiet#5002. Test item, control and reference materials, as well as test, control and reference diets were analysed for proximates, amino acids,

[30] Historical control data range was set on data from 35 groups of control SD rats, over 470 animals for each endpoint comparable in age, body weight, housing/environmental conditions, and given similar diets, spanning from the 5 years preceding and conducting the study under evaluation.

[31] Fisher exact test, two tailed, $p < 0.05$: 15% test diet p-value = 0.50; 30% test diet p-value = 0.30. For 15% & 30% test diets combined (5/32) p-value = 0.34.

minerals, mycotoxins and pesticides (including glufosinate and its metabolites 3-methyl-phosphinico-propionic acid (MPP) and *N*-acetyl-glufosinate (NAG)). In-life procedures and observations and terminal procedures were conducted in accordance to OECD TG 408 (1998). Mean, median, standard deviation, min and max were reported for all continuous endpoints for each group/sex and per period or time as appropriate.[32] No A5547-127 related mortality was observed during the study. One male from the control group was found dead on day 29 of the study and the cause of death was not determined. No test diet related clinical signs and ophthalmoscopic findings were observed. No statistically significant differences in mean body weight and cumulative body weight changes were observed in the GM group compared to the control group. A statistically significant increase in mean feed consumption as compared to controls was noted in the GM group at combined sex statistical analyses (+15.0%) and in female (+18.8%) during study weeks 9–10. The GMO Panel notes that this increase in feed intake is transient and not associated with differences in the body weight and cumulative body weight changes, and thus not adverse. No statistically significant differences were noted in functional observation batteries between the group given the test diet and the control group. A statistically significantly higher mean ambulatory count was noted in the test group males (+19.7%) during the 0–10 min interval and a statistically significantly lower mean ambulatory count was noted in the GM group females (−60.4%) during the 21–30 min interval when compared to the control group. The GMO Panel considers this finding is not adverse, being a single segment of the test battery and representing a relatively small change compared to controls. No statistically significant differences between the GM and the control group were noted regarding haematological and coagulation analysis, or at urinalysis. Higher aspartate transaminase was noted in females given the test diet as compared to controls. This finding is noted to be primarily associated with one animal, and it is not considered to be test diet-related. Lower thymus weight (absolute -15.0%, relative to final body weight −14.7%, relative to brain weight ratio −14.6%) was noted in females given the test diet when compared to the control group. This finding is not associated with histopathological changes in the thymus itself or in other lymphoid organs. Moreover, no haematological changes (i.e. changes in lymphocyte count) were noted in this group. The GMO Panel therefore considers this finding not to be adverse. No gross pathological findings related to the administration of the test diet were observed at necropsy. The microscopic examinations of selected organs and tissues did not identify relevant differences in the incidence and severity of the histopathological findings related to the administration of the test diet. Sporadic unilateral testes tubular atrophy was observed in a few rats across groups, up to severe in two rats from the test diet fed group. This finding is not statistically significant. The GMO Panel considers this finding a background condition, not related to the treatment with the test diet.

The GMO Panel notes that the applicant only tested one dose level. However, the dose tested was the highest possible without inducing nutritional imbalance according to current knowledge and in accordance with the limit test (OECD, 1998). This is considered not to compromise the study (EFSA, 2014).

The GMO Panel concludes that this study is in line with the requirements of Regulation (EU) 503/2013 and that no adverse effects were observed in rats after feeding diets including 30% of defatted toasted meal from soybean A5547-127 for 90 days.

3.6.4. Allergenicity

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

[32] A repeated measures model was set up first for the two genders separately and then pooling them. Model by sex included the treatment as a fixed effect, whereas block, time interval and interaction of time interval and diet were considered as random effects. Cage average value was included as the repeated measurement. The pooled analysis combining sexes was performed using a mixed effects model with diet, time, sex and double interaction of the single effects as fixed factors; random effects were the block and the cage, the latter treated as repeated measurement.

3.6.4.1. Assessment of allergenicity of newly expressed proteins[33]

The GMO Panel has previously evaluated the safety of the proteins DMO, CP4 EPSPS and PAT proteins individually, and no concerns on allergenicity were identified in the context of the applications assessed (Table 1). No new information on allergenicity of these proteins that might change the previous conclusions of the GMO Panel in the context of the GM events assessed has become available.[34] 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 three-event stack soybean are expected.

In addition, no information available on the structure or function of the newly expressed DMO, CP4 EPSPS and/or PAT proteins would suggest an adjuvant effect of these proteins in the three-event stack soybean, resulting in or increasing an eventual immunoglobulin E (IgE) response to a bystander protein.

3.6.4.2. Assessment of allergenicity of the GM plant products[35]

Soybean is considered a common allergenic food[36] (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, 2010c; Fernandez et al., 2013; Selb et al., 2017). The applicant also referred to the Kunitz trypsin inhibitor as a potential soybean allergen, which is an anti-nutrient and as such it is already assessed in the compositional analysis (Section 3.5.6). These allergens were selected based on the list of potential soybean allergens described in the pertinent OECD document (OECD, 2012) and a scientific rational supporting their selection was provided by the applicant and considered acceptable by the GMO Panel. No changes in the levels of endogenous allergens raising concern are identified by the GMO Panel.

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

3.6.5. Dietary exposure assessment to new constituents

In line with Regulation (EU) No 503/2013, the applicant provided dietary exposure estimates to DMO, CP4 EPSPS and PAT proteins newly expressed in soybean MON 87708 × MON 89788 × A5547-127. Dietary exposure was estimated based on protein expression levels reported in this application for the three-event stack soybean treated with the intended herbicides (Appendix A), 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.

[33] Dossier: Part II – Section 1.5.1, 1.5.3.

[34] The Panel notes that for DMO and CP4 EPSPS proteins an assessment regarding celiac disease was conducted by the applicant. The assessment identified no perfect or relevant partial matches with known celiac disease peptide sequences. It is pointed out that the requirements laid down in the recent EFSA guidance on allergenicity (2017) are not applicable to this dossier, as described in Section '1.5 Transition period'.

[35] Part II—Section 1.3.2, 1.3.3, 1.5.2 and additional information 25 August 2017.

[36] 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.

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 87708 × MON 89788 × A5547-127 treated with a combination of the intended herbicides[(a)]

Protein	Tissue/developmental stage	
	Seeds/R8 (μg/g dry weight and μg/g fresh weight[(b)])	Forage/R6 (μg/g dry weight)
DMO	28 ± 7/25 ± 6	28 ± 5
CP4 EPSPS	110 ± 16/97 ± 13	110 ± 16
PAT	38 ± 4/33 ± 4	67 ± 9

(a): Intended herbicides: dicamba, glyphosate and glufosinate-ammonium.
(b): Fresh weight values used to estimate human dietary exposure were calculated by multiplying the dry weight values (28, 110, 38 μg/g) by a dry weight correction factor of 0.88 to account for approximately 12% moisture content in the seeds.

3.6.5.1. Human dietary exposure[37]

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

For the purpose of estimating dietary exposure, the levels of newly expressed proteins in soybean MON 87708 × MON 89788 × A5547-127 seeds were derived from replicated field trial sites (five locations) in the 2015 US growing season. Mean values (fresh weight) are considered as the most representative to estimate dietary exposure (see Table 7). Since no specific consumption data were available on commodities containing soybean MON 87708 × MON 89788 × A5547-127, 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 Consumption Database (EFSA consumption database).[38] Soybean oil was excluded from the assessment since no proteins are expected to be present in the oil.

For acute dietary exposure estimations, the applicant used the protein content in the different soybean derived commodities to estimate the concentration of DMO, CP4 EPSPS and PAT proteins in the consumed foods. This is considered a conservative approach as no losses of newly expressed proteins are assumed during processing and all the protein content in the processed foods is considered as derived from the GM soybean. Summary statistics from the EFSA consumption database were used (accessed on June 2016).[39]

Acute dietary exposure in high consumers, within each dietary survey and age class, was estimated by summing the exposure derived from the 95th percentile consumption for the dominant food commodity[40] among consumers only and those exposures derived from the mean consumption of the remaining food categories in the total population (EFSA, 2015b). Among the young population, the highest acute exposure was estimated in other children (3–10 years old), with 286 μg/kg body weight (bw) per day, 97 μg/kg bw per day and 74 μg/kg bw per day for CP4 EPSPS, PAT and DMO proteins, respectively. In adults (18–65 years old), the highest acute exposure estimates were 496 μg/kg bw per day, 169 μg/kg bw per day and 128 μg/kg bw per day for CP4 EPSPS, PAT and DMO proteins, respectively. Most relevant food commodities in terms of contribution to the exposure were soya drink and meat imitates (textured soy protein).

The GMO Panel estimated chronic dietary exposure to CP4 EPSPS, PAT and DMO 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 19 European countries.[41] Different recipes and factors were considered to estimate the amount of soybean in the consumed commodities before assigning CP4 EPSPS, PAT and DMO proteins levels to

[37] Dossier: Part II – Section 2.4.
[38] http://www.efsa.europa.eu/en/data/food-consumption-data
[39] https://www.efsa.europa.eu/en/applications/gmo/tools
[40] Dominant food commodity refers to the food that will lead to the highest exposure among all consumed foods.
[41] Austria, Belgium, Bulgaria, Cyprus, the Czech Republic, Germany, Denmark, Spain, Finland, France, the United Kingdom, Greece, Hungary, Ireland, Italy, Latvia, the Netherlands, Romania, and Sweden.

the relevant commodities.[42] No losses in the newly expressed proteins during processing were considered. The 95th percentile chronic exposure was derived from the distribution of the individual dietary exposure estimates within each dietary survey and age class. In the young population, the highest 95th percentiles were estimated in other children, with 33.1 μg/kg bw per day, 11.3 μg/kg bw per day and 8.5 μg/kg bw per day for CP4 EPSPS, PAT and DMO proteins, respectively. Corresponding highest estimates were identified in the elderly and very elderly population (> 65 years) with 16.3 μg/kg bw day, 5.6 μg/kg bw day and 4.2 μg/kg bw day for CP4 EPSPS, PAT and DMO proteins, respectively. The main average contributors to the exposure in these dietary surveys were soya drink in the young population, and meat imitates and soya beans in the elderly and very elderly population.

A worst-case exposure scenario to cover dietary exposure to CP4 EPSPS, PAT and DMO proteins following the consumption of protein isolates (~90% protein) as protein supplements was investigated. In the absence of consumption data, high consumers of protein (95th percentile) in the European adult population (EFSA NDA Panel, 2012) were considered (up to 189 g/day). Assuming that all consumed protein was from soybean MON 87708 × MON 89788 × A5547-127 protein isolates, the estimated exposure to CP4 EPSPS, PAT and DMO in adults (body weight 70 kg) would be around 718 μg/kg bw day, 244 μg/kg bw day, and 185 μg/kg bw day, respectively.

3.6.5.2. Animal dietary exposure[43]

Animal dietary exposure to CP4 EPSPS, PAT and DMO 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 CP4 EPSPS, PAT and DMO proteins in soybean seeds and forage/silage were derived from replicated field trial sites (five locations) in the 2015 US growing season (Table 7). To estimate the mean newly expressed protein 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 newly expressed protein occur during processing.

Dietary exposure to CP4 EPSPS, PAT and DMO proteins in soybean MON 87708 × MON 89788 × A5547-127 following the consumption of soybean meal was provided by the applicant across different animal species (i.e. broiler, finishing pig and lactating 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 was as follows:

- To CP4 EPSPS protein, 3,976 μg/kg bw per day in broiler chickens, 1354 μg/kg bw per day in dairy cattle and 1,267 μg/kg bw per day in finishing pig.
- To PAT protein, 1,373 μg/kg p bw per day in broiler chickens, 468 μg/kg bw per day in dairy cattle and 438 μg/kg bw per day in finishing pig.
- To DMO protein, 1,012 μg/kg bw per day in broiler, 345 μg/kg bw per day in dairy cattle and 323 μg/kg bw per day in finishing pig.

The GMO Panel estimated dietary exposure to CP4 EPSPS, PAT and DMO proteins in lactating dairy cows 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 lactating dairy cows was 847 μg/kg bw per day, 516 μg/kg bw per day and 216 μg/kg bw per day for CP4 EPSPS, PAT and DMO proteins, respectively.

3.6.6. Nutritional assessment of endogenous constituents

The intended traits of the three-event stack soybean are herbicide tolerance, with no intention to alter nutritional parameters. However, ADF, total fat and behenic acid were significantly different from its conventional counterpart and showed a lack of equivalence with the set of non-GM reference varieties (Section 3.5.6). The biological relevance of these compounds, the role of soybean as contributor to their total intake and the magnitude and direction of the observed changes were considered during the nutritional assessment.

[42] Example: 100 grams of Tofu are made with approximately 26 grams of soy beans; this would result in 25.2 μg of CP4 EPSPS per gram of tofu as compared to 97 μg/g in the soy beans.
[43] Dossier: Part II – Section 2.3.

3.6.6.1. Human nutrition

In the context of human nutrition, fibre is referred to as dietary fibre, which primarily includes non-starch polysaccharides (mainly cellulose, hemicelluloses, pectins, and other hydrocolloids) and lignin (EFSA NDA Panel, 2010). Therefore, the observed increase (~ 11%) in ADF (cellulose and lignin) implies an increase of dietary fibre. Dietary fibre is present in certain soybean derived foods (e.g. soybean flour), while it is almost absent in other products such as tofu and soya milk. Although evidences link high consumption of fibre (above 25 grams per day) with health benefits (EFSA NDA Panel, 2010), foods from soybean are, overall, not major contributors to total dietary fibre intake. Therefore, the nutritional impact of the increase in ADF is not considered relevant for human nutrition. The decrease in total fat (~ 3%) is not considered relevant for human nutrition, also because no significant differences in the profile of FA beyond behenic acid were observed. Behenic acid is a saturated FA (22:0) present in vegetable oils in very small amounts (< 1% total FA) although levels around 3% of the total FA have been reported in peanut oil (Ozcan and Seven, 2003). Considering the very low levels of behenic acid in soybean (< 1% total FA) and the observed decrease as compared to the conventional counterpart, the GMO Panel concludes that the nutritional impact of foods derived from soybean MON 87708 × MON 89788 × A5547-127 is similar to that of foods derived from its conventional counterpart and non-GM reference varieties.

3.6.6.2. Animal nutrition

Animal complete diets are balanced, i.e. ADF and total fat from feed ingredients are taken into account to meet animal nutritional requirements. Considering the very low levels of behenic acid in soybean (< 1% total FA) and the observed decrease as compared to the conventional counterpart, the nutritional impact in feeds is considered negligible.

3.6.6.3. Conclusion on human and animal nutrition

Based on the current knowledge on the biological role of the compounds assessed, the magnitude and direction of the changes identified, and the relevance of soybean as contributor to the intake of these compounds, the GMO Panel concludes that the nutritional impact of foods and feeds from the three-event stack soybean is expected to be the same as those from its conventional counterpart and non-GM reference varieties.

3.6.7. Conclusion of the food and feed safety assessment

The proteins DMO, CP4 EPSPS and PAT newly expressed in soybean MON 87708 × MON 89788 × A5547-127 do not raise safety concerns for human and animal health. Interactions between these newly expressed proteins raising food and feed safety concerns (toxicology, allergenicity and adjuvanticity) are not expected. There is no evidence that the genetic modification might change the overall allergenicity of the three-event stack soybean. The nutritional impact of the three-event stack soybean foods and feeds is expected to be the same as that of foods and feeds derived from its conventional counterpart and non-GM reference varieties. The GMO Panel concludes that the three-event stack soybean, as described in this application, is as safe as and nutritionally equivalent to its conventional counterpart and the non-GM reference varieties tested.

3.7. Environmental risk assessment[44]

Considering the scope of application EFSA-GMO-NL-2016-135, which excludes cultivation, the environmental risk assessment (ERA) of the three-event stack soybean 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 the three-event stack soybean seeds during transportation and/or processing (EFSA GMO Panel, 2010b).

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).

[44] Dossier: Part II – Section 5; additional information: 31/5/2018.

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 the three-event stack soybean will provide a selective advantage to soybean plants, except when they are exposed to glyphosate-, dicamba- and/or glufosinate-containing herbicides. However, this fitness advantage will not allow the GM plant to overcome other biological and abiotic factors (described above) limiting plant's persistence and invasiveness. Therefore, the presence of the intended traits will not affect the persistence and invasiveness of the GM plant.

In conclusion, the GMO Panel considers it very unlikely that the three-event stack soybean 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 seeds of the three-event stack soybean.

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 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 HR.

The updated bioinformatic analysis for event MON 87708 revealed two elements with sufficient length and sequence identity with bacterial genes in databases: the *dmo* gene from *S. maltophilia*, and the left border sequence from the *Agrobacterium tumefaciens* Ti plasmid. Considering that these sequences do not occur in the same bacterial species, there is no indication for facilitated double HR from MON 87708 confirming the conclusions of a previous EFSA GMO Panel Scientific Opinion (EFSA GMO Panel, 2015b).

The updated bioinformatic analysis for event MON 89788 revealed no homology with known DNA sequences from bacteria which would facilitate HR confirming the conclusions of a previous EFSA GMO Panel Scientific Opinion (EFSA GMO Panel, 2015b).

The potential for HGT of event A5547-127 has been recently assessed by the GMO Panel in the context of application EFSA-GMO-NL-2013-120 (EFSA GMO Panel, 2017). The assessment, based on updated bioinformatic analysis, confirmed that double HR could occur between the non-functional *bla* gene fragments of event A5547-127, with a chromosomally located *bla* gene, leading to a chromosomally inserted *pat* gene. Due to its plant codon optimisation, it is expected that the newly acquired *pat* gene would not provide a selective advantage to bacterial recipients. Confirming the previous conclusion of the GMO Panel, no risk was identified for HGT of the recombinant DNA derived from event A5547-127.

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 three-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 87708 × MON 89788 × A5547-127 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 87708 × MON 89788 × A5547-127 seeds are processed in the countries of production or in ports of importation.

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

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

The potential of spilled soybean seeds to establish, grow and produce pollen is extremely low and transient (see Section 3.7.1). Therefore, the likelihood/frequency of cross-pollination between occasional feral GM soybean plants resulting from seed spillage, and weedy or cultivated soybean plants is also considered extremely low. Even if cross-pollination would occur, the GMO Panel is of the opinion that the likelihood of environmental effects as a consequence of the spread of genes from occasional feral GM soybean plants in Europe will not differ from that of conventional soybean varieties for the reasons given in Section 3.7.1.

3.7.3. Interactions of the GM plant with target organisms

Taking the scope of application EFSA-GMO-NL-2016-135 (no cultivation) and thus the absence of target organisms into account, potential interactions of occasional feral three-event stack soybean plants arising from seed import spills with target organisms 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 three-event stack soybean 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 three-event stack soybean with non-target organisms are not considered to raise any environmental safety concern.

3.7.5. Interactions with abiotic environment and biogeochemical cycles

Given that environmental exposure to spilled seeds or occasional feral three-event stack soybean 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 to raise any environmental safety concern.

3.7.6. Conclusion of the environmental risk assessment

The GMO Panel concludes that it is unlikely that the three-event stack soybean would differ from conventional soybean varieties in its ability to persist under European environmental conditions. Considering the scope of application EFSA-GMO-NL-2016-135, interactions of occasional feral three-event stack soybean plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from the three-event stack soybean to bacteria does not indicate a safety concern. Therefore, 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 the

three-event stack soybean 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

There was no indication that food/feed products derived from the three-event stack soybean are less safe or nutritious than those derived from the non-GM conventional counterpart. Furthermore, the overall intake or exposure is not expected to change because of the introduction of the three-event stack soybean into the market. Therefore, the GMO Panel considers that Post-Market Monitoring (PMM) of food and feed from the three-event stack soybean is not necessary.

3.8.2. Post-market environmental monitoring[45]

The objectives of a PMEM plan, according to Annex VII of Directive 2001/18/EC, are: (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the ERA are correct; and (2) 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 the three-event stack soybean, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for the three-event stack soybean 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 the three-event stack soybean. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

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

3.8.3. Conclusion on post-market monitoring

No PMM 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 three-event stack soybean.

3.9. Overall conclusions

The GMO Panel was asked to carry out a scientific assessment of soybean MON 87708 × MON 89788 × A5547-127 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 87708, MON 89788 and A5547-127 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, allergenicity and nutritional assessment indicate that the combination of the single soybean events and of the newly expressed proteins in the three-event stack soybean does not give rise to food/feed safety and nutritional concerns. The GMO Panel concludes that the three-event stack soybean, as described in this application, is as safe as and nutritionally equivalent to its conventional counterpart 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 three-event stack soybean into the environment.

[45] Dossier: Part II – Section 6.

Based on the relevant publications identified through the literature searches, the GMO Panel did not identify any safety issues pertaining to the intended uses of soybean MON 87708 × MON 89788 × A5547-127. In the context of annual PMEM reports, the applicant should 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 soybean MON 87708 × MON 89788 × A5547-127.

Given the absence of safety concerns for foods and feeds from the soybean MON 87708 × MON 89788 × A5547-127, 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 three-event stack soybean.

In conclusion, the GMO Panel considers that soybean MON 87708 × MON 89788 × A5547-127, as described in this application, is as safe as its conventional counterpart and the tested non-GM reference varieties with respect to potential effects on human and animal health and the environment.

Documentation provided to EFSA

1) Letter from the Competent Authority of Netherlands received 03 November 2016 concerning a request for authorisation for the placing on the market of soybean MON 87708 × MON 89788 × A5547-127 (reference EFSA-GMO-NL-2016-135) submitted in accordance with Regulation (EC) No 1829/2003 by Monsanto Europe S.A./N.V.
2) Application EFSA-GMO-NL-2016-135 validated by EFSA, 19 January 2017.
3) Request for supplementary information to the applicant, 25 January 2017.
4) Receipt of supplementary information from the applicant, 30 January 2017.
5) Request for supplementary information to the applicant, 23 February 2017.
6) Receipt of supplementary information from the applicant, 21 April 2017.
7) Request for supplementary information to the applicant, 11 May 2017.
8) Receipt of supplementary information from the applicant, 16 June 2017.
9) Request for supplementary information to the applicant, 26 June 2017.
10) Request for supplementary information to the applicant, 12 July 2017.
11) Receipt of supplementary information from the applicant, 17 July 2017.
12) Receipt of supplementary information from the applicant, 28 August 2017.
13) Request for supplementary information to the applicant, 15 February 2018.
14) Receipt of supplementary information from the applicant, 16 March 2018.
15) Receipt of spontaneous information from the applicant, 27 April 2018.
16) Receipt of spontaneous information from the applicant, 31 May 2018.
17) Receipt of supplementary information from the applicant, 04 July 2018.
18) Request for supplementary information to the applicant, 18 July 2018.
19) Receipt of supplementary information from the applicant, 14 August 2018.
20) Request for supplementary information to the applicant, 30 August 2018.
21) Request for supplementary information to the applicant, 13 September 2018.
22) Receipt of supplementary information from the applicant, 28 September 2018.
23) Receipt of supplementary information from the applicant, 28 September 2018.
24) Receipt of supplementary information from the applicant, 06 November 2018
25) Request for supplementary information to the applicant, 08 November 2018.
26) Receipt of supplementary information from the applicant, 13 November 2018.
27) Request for supplementary information to the applicant, 29 November 2018.
28) Receipt of supplementary information from the applicant, 12 December 2018
29) Request for supplementary information to the applicant, 21 December 2018.
30) Receipt of supplementary information from the applicant, 22 January 2019.
31) Receipt of spontaneous information from the applicant, 20 March 2019.
32) Receipt of spontaneous information from the applicant, 02 April 2019.

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Abbreviations

ADF	acid detergent fibre
BBCH	Biologische Bundesanstalt, Bundessortenamt and Chemical Industry
bw	body weight
DMO	dicamba mono-oxygenase
ELISA	enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	environmental risk assessment
FA	fatty acid
GLP	good laboratory practice
GM	genetically modified
GMO	genetically modified organism
GMO Panel	EFSA Panel on Genetically Modified Organisms
HCD	historical control data
HGT	horizontal gene transfer
HR	homologous recombination
IgE	immunoglobulin E
LUC	large unstained cells
MPP	3-methyl-phosphinico-propionic acid
MS	Member States
NAG	*N*-acetyl-glufosinate
NDF	neutral detergent fibre
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PAT	phosphinothricin acetyltransferase
PCR	polymerase chain reaction
PMEM	post-market environmental monitoring
PMM	post-market monitoring
PMI	phosphomannose isomerase
UTR	untranslated region
WBC	white blood cell

Appendix A – Protein expression data

Mean, standard deviation and range of protein levels (μg/g dry weight) from soybean MON 87708 × MON 89788 × A5547-127 (treated with dicamba, glyphosate and glufosinate-ammonium), MON 87708 (treated with dicamba), MON 89788 (treated with glyphosate) and A5547-127 (treated with glufosinate-ammonium) from a field trial performed across five locations in USA in 2015 (n = 20).

Protein	Event(s)	Leaf (V3–V4)	Leaf (R6)	Forage (R6)	Root (R6)	Seed (R8)
DMO	**MON 87708 × MON 89788 × A5547-127**	12.4[(a)] ± 4.4[(b)] (5.7–20)[(c)]	46 ± 18 (23–86)	28 ± 5.3 (20–37)	4.4 ± 2.8 (1.3–11)	28 ± 7.3 (21–57)
	MON 87708	12 ± 4.0 (6.1–18)	48 ± 27 (19–110)	29 ± 5.4 (20–40)	4.6 ± 3.1 (1.1–12)	26 ± 4.1 (11–35)
CP4 EPSPS	**MON 87708 × MON 89788 × A5547-127**	170 ± 38 (100–240)	170 ± 43 (74–270)	110 ± 16 (91–150)	22 ± 9.8 (8.1–43)	110 ± 16 (78–140)
	MON 89788	180 ± 57 (96–350)	180 ± 38 (130–280)	130 ± 11 (100–150)	53 ± 14 (35–88)	110 ± 11 (95–140)
PAT	**MON 87708 × MON 89788 × A5547-127**	82 ± 21 (39–130)	100 ± 37 (40–180)	67 ± 9.4 (50–86)	19 ± 6.8 (9.0–31)	38 ± 4.4 (30–45)
	A5547-127	66 ± 11 (43–85)	110 ± 20 (80–150)	54 ± 9.7 (37–71)	23 ± 8.2 (9.8–38)	37 ± 5.6 (24–46)

DMO: dicamba mono-oxygenase; EPSPS: 5-enolpyruvylshikimate-3-phosphate synthase; PAT: phosphinothricin acetyltransferase.
(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 87708 × MON 89788 × A5547-127

Study identification	Title
MSL0027645	Phenotypic Evaluation and Environmental Interactions of Soybean MON 87708 × MON 89788 × A5547-127 in 2015 U.S. Field Trials
MSL0027453	Analyses of Minerals and B Vitamins of Soybean Seed from MON 87708 × MON 89788 × A5547-127 Grown in the United States in 2015
MSL0027882	Comparison of Gly m 4 Expression Levels from MON 87708 × MON 89788 × A5547-127 and Conventional Soybeans
MSL0027424	Southern Blot Analyses to Confirm the Presence of MON 87708, MON 89788 and A5547-127 in the Combined Trait Soybean Product MON 87708 × MON 89788 × A5547-127