SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 1 March 2017

doi: 10.2903/j.efsa.2017.4744

Scientific opinion on application EFSA-GMO-NL-2013-120 for authorisation of genetically modified soybean FG72 × A5547-127 for food and feed uses, import and processing submitted in accordance with Regulation (EC) No 1829/2003 by Bayer CropScience LP and M.S. Technologies LLC

EFSA Panel on Genetically Modified Organisms (GMO), Hanspeter Naegeli, Andrew Nicholas Birch, Josep Casacuberta, Adinda De Schrijver, Mikołaj Antoni Gralak, Philippe Guerche, Huw Jones, Barbara Manachini, Antoine Messéan, Elsa Ebbesen Nielsen, Fabien Nogué, Christophe Robaglia, Nils Rostoks, Jeremy Sweet, Christoph Tebbe, Francesco Visioli, Jean-Michel Wal, Andrea Gennaro, Franco Maria Neri and Irina Olaru

Abstract

In this opinion, the EFSA Panel on Genetically Modified Organisms (GMO) assesses the two-event stack soybean FG72 × A5547-127 for food and feed uses, import and processing. The EFSA GMO Panel previously assessed the two single events combined to produce the two-event stack soybean FG72 × A5547-127 and did not identify safety concerns. No new data on the single events, leading to modification of the original conclusions on their safety, were identified. The molecular, agronomic, phenotypic and compositional data on soybean FG72 × A5547-127 did not give rise to safety concerns and no reason to expect interactions between the single events impacting on the food and feed safety of the two-event stack soybean was identified. Although the EFSA GMO Panel cannot conclude on forage composition, soybean forage is not expected to be imported in a significant amount for use as feed. Considering the routes of exposure and limited exposure levels, the EFSA GMO Panel concludes that soybean FG72 × A5547-127 would not give rise to safety concerns in the event of accidental release of viable seeds into the environment. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of soybean FG72 × A5547-127. The EFSA GMO Panel concludes that soybean FG72 × A5547-127 is as safe as the non-genetically modified (GM) comparator and non-GM soybean reference varieties with respect to potential effects on human and animal health and the environment.

Keywords: GMO, soybean (*Glycine max*), FG72 × A5547-127, Regulation (EC) 1829/2003, HPPD W336, 2mEPSPS, PAT, import and processing

Requestor: European Commission

Question number: EFSA-Q-2013-01032

Correspondence: GMO@efsa.europa.eu

Panel members: Andrew Nicholas Birch, Josep Casacuberta, Adinda De Schrijver, Achim Gathmann, Mikolaj Antoni Gralak, Philippe Guerche, Huw Jones, Barbara Manachini, Antoine Messéan, Hanspeter Naegeli, Elsa Ebbesen Nielsen, Fabien Nogué, Christophe Robaglia, Nils Rostoks, Jeremy Sweet, Christoph Tebbe, Francesco Visioli and Jean-Michel Wal.

Acknowledgements: The Panel wishes to thank the following for the support provided to this scientific opinion: the members of the Working group on Molecular Characterisation, Food and Feed Safety Assessment and Environmental Risk Assessment for the preparatory work on this scientific opinion. The Panel wishes to acknowledge all European competent institutions, Member State bodies and other organisations that provided data for this scientific opinion.

Suggested citation: EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Birch AN, Casacuberta J, De Schrijver A, Gralak MA, Guerche P, Jones H, Manachini B, Messéan A, Nielsen EE, Nogué F, Robaglia C, Rostoks N, Sweet J, Tebbe C, Visioli F, Wal JM, Gennaro A, Neri FM and Olaru I, 2017. Scientific opinion on application EFSA-GMO-NL-2013-120 for authorisation of genetically modified soybean FG72 × A5547-127 for food and feed uses, import and processing submitted in accordance with Regulation (EC) No 1829/2003 by Bayer CropScience LP and M.S. Technologies LLC. EFSA Journal 2017;15(4):4744, 23 pp. doi:10.2903/j.efsa.2017.4744

ISSN: 1831-4732

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

Summary

Following the submission of application EFSA-GMO-NL-2013-120 under Regulation (EC) No 1829/2003 from Bayer CropScience LP and M.S. Technologies LLC, the Panel on Genetically Modified Organisms of the European Food Safety Authority (GMO Panel) was asked to deliver a scientific opinion on the safety of herbicide-tolerant genetically modified (GM) soybean FG72 × A5547-127. The scope of application EFSA-GMO-NL-2013-120 is for food and feed uses, import and processing, but excludes cultivation within the European Union (EU).

The single soybean events FG72 (producing HPPD W336 and 2mEPSPS) and A5547-127 (producing PAT) were assessed previously 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 two single soybean events, since the publication of the respective scientific opinions. Consequently, the GMO Panel considers that its previous conclusions on the safety of the single soybean events remain valid.

In accordance to the GMO Panel guidance documents applicable to this application, 'for GM plants containing a combination of transformation events (stacked events) the primary concern for risk assessment is to establish that the combination of events is stable and that no interactions between the stacked events, that may raise safety concerns compared to the single events, occur. The risk assessment of GM plants containing stacked events focuses on issues related to: (a) stability of the inserts, (b) expression of the introduced genes and their products and (c) potential synergistic or antagonistic effects resulting from the combination of the events'.

In delivering this Scientific Opinion, the GMO Panel considered the data available on the two-event stack soybean, the scientific comments submitted by the Member States and the relevant scientific literature. Soybean FG72 × A5547-127 was produced by conventional crossing of soybean FG72 and soybean A5547-127 and no new genetic modifications were involved. Soybean FG72 × A5547-127 is tolerant to glyphosate-, isoxaflutole- and glufosinate-ammonium-based herbicides. The scientific evaluation of the risk assessment included molecular characterisation of the inserted DNA and analysis of the expression of the corresponding proteins. An evaluation of the comparative analyses of the compositional, agronomic and phenotypic characteristics was undertaken, and the safety of the newly expressed proteins and the whole food/feed was evaluated with respect to potential toxicity, allergenicity and nutritional wholesomeness. An evaluation of environmental impacts and the post-market environmental monitoring (PMEM) plan was also undertaken.

The molecular data establish that the transformation events stacked in soybean FG72 × A5547-127 retained their integrity. Comparison of the levels of the newly expressed proteins between the stack and the respective single events did not reveal an interaction that manifests at protein expression level. From the molecular characterisation, no indications of interactions between the events based on the biological functions of the newly expressed proteins were identified.

Based on the agronomic and phenotypic characteristics of soybean FG72 × A5547-127 under the tested conditions (not treated and treated with the intended herbicides), some differences were observed in soybean FG72 × A5547-127 compared with the non-GM comparator. These differences were further assessed and found not to affect the ability of soybean FG72 × A5547-127 to survive until subsequent seasons, or to establish occasional feral plants under European environmental conditions. The GMO Panel concludes that none of the differences identified in seed composition and agronomic/phenotypic characteristics between soybean FG72 × A5547-127 and the non-GM comparator needs further assessment regarding food and feed safety. Although the GMO Panel cannot conclude on forage composition, soybean forage is not expected to be imported in a significant amount for use as feed.

The proteins newly expressed in soybean FG72 × A5547-127 do not raise safety concerns for human and animal health, since no adverse effects were observed, no structural similarities to known toxins were detected, and no reasons were identified that the presence of the three proteins in combination would result in effects different from those of the individual proteins. Similarly, the GMO Panel did not identify indications of safety concerns regarding allergenicity with the individual newly expressed proteins or their mixture in soybean FG72 × A5547-127, or regarding potential changes of its overall allergenicity. Soybean FG72 × A5547-127 is expected to be as nutritious as the non-GM comparator and non-GM soybean reference varieties.

Considering the scope of this application, the environmental risk assessment is concerned with the accidental release into the environment of viable soybean FG72 × A5547-127 grains (i.e. during transport and/or processing), and with the exposure of bacteria to recombinant DNA in the

gastrointestinal tract of animals fed GM material and those present in environments exposed to their faecal material (manure and faeces).

In the case of accidental release into the environment of viable seeds of soybean FG72 × A5547-127, there are no indications of an increased likelihood of establishment and spread of feral soybean FG72 × A5547-127 plants, unless these plants are exposed to isoxaflutole-, glyphosate- and/or glufosinate-ammonium-based herbicides. However, the possible exposure to these herbicides would not result in different environmental impacts compared to conventional soybean. Considering the scope of application EFSA-GMO-NL-2013-120, interactions with the biotic and abiotic environment were not considered to be a relevant issue. Risks associated with an unlikely but theoretically possible horizontal gene transfer of recombinant DNA from the two-event stack soybean to bacteria have not been identified.

The GMO Panel considers that post-market monitoring of soybean FG72 × A5547-127 products is not necessary. The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of soybean FG72 × A5547-127 and the GMO Panel guidelines on the PMEM of GM plants.

In conclusion, the GMO Panel is of the opinion that soybean FG72 × A5547-127, as described in this application, is as safe as the non-GM comparator and non-GM soybean reference varieties with respect to potential effects on human and animal health and the environment.

Table of contents

1. Introduction

1.1. Background

On 10 December 2013, the European Food Safety Authority (EFSA) received from the Competent Authority of The Netherlands application EFSA-GMO-NL-2013-120, for authorisation of genetically modified (GM) soybean FG72 × A5547-127 submitted by Bayer CropScience LP and M.S. Technologies LLC within the framework of Regulation (EC) No 1829/2003 for food and feed uses, import and processing.[1]

After receiving the application EFSA-GMO-NL-2013-120 and in accordance with Articles 5(2)(b) and 17(2)(b) of Regulation (EC) No 1829/2003, EFSA informed Member States and the European Commission, and made the summary of the application available to the public on the EFSA website. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of Regulation (EC) No 1829/2003. On 17 January 2014 and on 2 February 2015, EFSA received additional information (requested on 19 December 2013 and on 28 February 2014). On 23 February 2015, EFSA declared the application valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the European Commission, and consulted nominated risk assessment bodies of Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC[2] following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Member States had 3 months after the date of receipt of the valid application (i.e. until 26 November 2015[3]) to make their opinion known.

The EFSA Panel on Genetically Modified Organisms (GMO Panel) carried out an evaluation of the scientific risk assessment of soybean FG72 × A5547-127 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003.

The GMO Panel requested additional information from the applicant on 7 July 2015, 18 September 2015, 28 February 2016, 23 March 2016, 15 June 2016, 28 June 2016, 27 September 2016 and 5 December 2016. The applicant provided the requested information on 20 August 2015, 19 November 2015, 7 April 2016, 28 April 2016, 12 August 2016, 8 November 2016 and 30 January 2017.

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

In giving its scientific opinion to the European Commission, the Member States and the applicant, and in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003, EFSA has endeavoured to respect a time limit of 6 months from the acknowledgement of the valid application. As additional information was requested by the EFSA GMO Panel, the time limit of 6 months was extended accordingly, in line with Articles 6(1), 6(2), 18(1) and 18(2) of Regulation (EC) No 1829/2003.

According to Regulation (EC) No 1829/2003, this scientific opinion is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation and thus will be part of the EFSA overall opinion in accordance with Articles 6(5) and 18(5).

1.2. Terms of Reference as provided by the requestor

The GMO Panel was requested to carry out a scientific risk assessment of soybean FG72 × A5547-127 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003.

Where applicable, any conditions or restrictions which should be imposed on the placing on the market and/or specific conditions or restrictions for use and handling, including post-market monitoring requirements based on the outcome of the risk assessment and, in the case of genetically modified organisms (GMOs) or food/feed containing or consisting of GMOs, conditions for the protection of particular ecosystems/environment and/or geographical areas should be indicated in accordance with Articles 6(5)(e) and 18(5)(e) of Regulation (EC) No 1829/2003.

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed.

[2] 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.

[3] The Member States' commenting period of application EFSA-GMO-NL-2013-120 was suspended until the clock of the application was re-started following the adoption of the Scientific Opinions of application EFSA-GMO-BE-2011-98 (GM soybean FG72).

The EFSA GMO Panel was not requested to give an opinion on information required under Annex II to the Cartagena Protocol. Furthermore, the EFSA GMO Panel did not consider proposals for labelling and methods of detection (including sampling and the identification of the specific transformation event in the food/feed and/or food/feed produced from it), which are matters related to risk management.

2. Data and methodologies

2.1. Data

In delivering its scientific opinion, the GMO Panel took into account application EFSA-GMO-NL-2013-120, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications.

2.2. Methodologies

The GMO Panel carried out a scientific risk assessment of soybean FG72 × A5547-127 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. The EFSA GMO Panel took into account the appropriate principles described in its guidelines for the risk assessment of GM plants and derived food and feed (EFSA GMO Panel, 2011a), for the environmental risk assessment (ERA) of GM plants (EFSA GMO Panel, 2010a) and for the post-market environmental monitoring (PMEM) of GM plants (EFSA GMO Panel, 2011b).

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

3. Assessment

3.1. Introduction

Application EFSA-GMO-NL-2013-120 covers the two-event stack soybean FG72 × A5547-127 produced by conventional crossing of events FG72 and A5547-127. The scope of this application is for food and feed uses, import and processing, but excludes cultivation within the European Union (EU).

Soybean FG72 × A5547-127 (unique identifier MST-FGØ72-2 × ACS-GMØØ6-4) was developed to confer tolerance to isoxaflutole- (5-cyclopropylisoxazol-4-yl 2-mesyl-4-trifluoromethylphenyl ketone), glyphosate- (*N*-(phosphonomethyl) glycine) and glufosinate (L-phosphinothricin) ammonium-based herbicides. Tolerance to these herbicides is achieved by expression of the HPPD W336 (4-hydroxyl phenyl-pyruvate-dioxygenase), 2mEPSPS (5-enolpyruvylshikimate-3-phosphate synthase) and PAT (phosphinothricin acetyl-transferase) proteins, respectively.

Soybean FG72 × A5547-127 was produced by conventional crossing of the two single soybean events FG72 and A5547-127, which have been previously assessed (see Table 1) on the basis of experimental data. No concerns for human and animal health or environmental safety were identified.

Table 1: Single soybean events already assessed by the EFSA GMO Panel

Event	Application	EFSA Scientific Opinion
FG72	EFSA-GMO-BE-2011-98	EFSA GMO Panel (2015)
A5547-127	EFSA-GMO-NL-2008-52	EFSA GMO Panel (2011c)

The EFSA guidance establishes the principle that 'For GM plants containing a combination of transformation events (stacked events) the primary concern for risk assessment is to establish that the combination of events is stable and that no interactions between the stacked events, that may raise safety concerns compared to the single events, occur. The risk assessment of GM plants containing stacked events focuses on issues related to: (a) stability of the inserts, (b) expression of the introduced genes and their products and (c) potential synergistic or antagonistic effects resulting from the combination of the events' (EFSA GMO Panel, 2011a).

3.2. Updated information on single events

Since the publication of the scientific opinions on the single soybean events by the GMO Panel (EFSA GMO Panel, 2011c, 2015), no safety issue pertaining to the two single events has been reported by the applicant.

Updated bioinformatic analyses on the junction regions for events FG72 and A5547-127 confirmed that no known endogenous genes were disrupted by any of the inserts.[4]

Updated bioinformatic analyses of the amino acid sequence of the newly expressed HPPD W336, 2mEPSPS and PAT proteins revealed no significant similarities to toxins and allergens.[5] In addition, updated bioinformatics analyses of the newly created Open Reading Frames (ORFs) within the inserts and at their junctions, did not indicate significant similarities to toxins and allergens either.[5]

In order to assess the possibility for horizontal gene transfer (HGT) by homologous recombination (HR), the applicant performed a sequence identity analysis for events FG72 and A5547-127. The assessment of these data and the potential consequences of plant-to-bacteria gene transfer are described in Section 3.6.2.2.

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. Molecular characterisation

Possible interactions affecting the integrity of the events, protein expression level or the biological functions conferred by the individual inserts are considered.

3.3.1. Genetic elements and their biological functions[6]

Soybean FG72 × A5547-127 was obtained by conventional crossing of soybean FG72 and soybean A5547-127. The structure of the inserts introduced into soybean FG72 and A5547-127 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 FG72 × A5547-127 are summarised in Table 3.

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

Table 2: Genetic elements in the expression cassettes of the events stacked in soybean FG72 × A5547-127

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
FG72	*Ph4a748 ABBC* (*Arabidopsis thaliana*)*	5′tev (Tobacco etch virus)	TPotp Y (*Zea mays* and *Helianthus annuus*)	*hppdPfW336* (*Pseudomonas fluorescens*)	*nos* (*Agrobacterium tumefaciens*)
	Ph4a748 (*A. thaliana*)	–	TPotp C (*Z. mays* and *H. annuus*)	*2mepsps* (*Z. mays*)	3′histonAt (*A. thaliana*)
A5547-127	p35S (CaMV)	–	–	*pat* (*Streptomyces viridochromogenes*)	*t35S* (CaMV)

CaMV: cauliflower mosaic virus; UTR: untranslated region.
(*): Source of genetic information.
–: When no element was specifically introduced to optimise expression.

[4] Additional information: 30/01/2017.
[5] Additional information: 12/08/2016.
[6] Dossier: Part II – Section A.2.

Table 3: Characteristics and intended effects of the events stacked in soybean FG72 × A5547-127

Event	Protein	Donor organism and biological function	Intended effects in GM plant
FG72	HPPD W336	Based on a gene from *Pseudomonas fluorescens* strain A32. 4-hydroxyl phenyl-pyruvate-dioxygenase (HPPD) is involved in the biosynthesis of homogentisate in plants	The bacterial HPPD was modified by substituting one amino acid, which reduces sensitivity to isoxaflutole herbicides. The expression of HPPD W336 in soybean confers tolerance to isoxaflutole (Boudec et al., 2001)
	2mEPSPS	Based on a gene from *Zea mays*. 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) is an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Herrmann, 1995)	The amino acid sequence of the maize EPSPS enzyme was modified to render the maize tolerant to glyphosate. Expression of 2mEPSPS confers tolerance to glyphosate-based herbicides (Lebrun et al., 1997)
A5547-127	PAT	Based on a gene from *Streptomyces viridochromogenes* Phosphinothricin-acetyl-transferase (PAT) enzyme acetylates phosphinothricin, rendering it inactive (Strauch et al., 1993)	Expression of PAT in soybean A5547-127 confers tolerance to glufosinate ammonium-based herbicides

3.3.2. Integrity of the events in soybean FG72 × A5547-127[7]

The genetic stability of the inserted DNA over multiple generations in single soybean events FG72 and A5547-127 was demonstrated previously (EFSA GMO Panel, 2011c, 2015). Integrity of these events in soybean FG72 × A5547-127 was demonstrated by Southern analyses.

3.3.3. Information on the expression of the inserts[8]

Plants were grown in 2012-2013 (three locations, four replicate plots) under field conditions in Brazil. The levels of the 2mEPSPS, HPPD W336 and PAT proteins in the two-event stack soybean and the two single events were analysed by enzyme-linked immunosorbent assay (ELISA) only in grains. Levels of 2mEPSPS and PAT were similar in FG72 × A5547-127, FG72 and A5547-127. Overall levels of HPPD W336 for both FG72 × A5547-127 and FG72 were low; levels of HPPD W336 in FG72 × A5547-127 were below the method's limit of quantification, whereas in the single they were quantifiable. Following a clarification request of the GMO Panel, the applicant provided a new data set, based on a field trial conducted in 2014 (three locations, four replicate plots), under field conditions in the USA.[9] Protein levels of the 2mEPSPS, HPPD W336 and PAT in the two-event stack soybean and the two single events were analysed by ELISA in leaf, root, flower, forage and grain, collected from treated or non-treated plants. Levels reported from this field trial were all above the method's limit of quantification, with one exception (a root sample from FG72 × A5547-127 for HPPD W336). Both data sets were taken into account by the GMO Panel for the assessment of the expression of the insert. Seed is the product predominantly imported in the EU. The highest mean values of the protein levels in seed in any of the two field trials are shown in Table 4.

Table 4: Means, standard deviation and ranges of protein levels (μg/g dry weight) in soybean FG72 × A5547-127 seed

Protein	Seed
HPPD W336	$1.26^{1,(a)} \pm 0.25^{(b)}$ (0.89–1.60)$^{(c)}$
2mEPSPS	$245.21^{2} \pm 31.61$ (184.59–282.07)
PAT	$19.6^{3} \pm 5.2$ (10.9–26.8)

HPPD: 4-hydroxyl phenyl-pyruvate-dioxygenase; EPSPS: 5-enolpyruvylshikimate-3-phosphate synthase; PAT: phosphinothricin acetyl-transferase.
[1]Not treated, USA; [2]not treated, USA; [3]treated, Brazil.
(a): Mean.
(b): Standard deviation.
(c): Range.

[7] Dossier: Part II – Section A.2.2.2.
[8] Dossier: Part II – Section A.2.2.3.
[9] Additional information: 7/4/2016.

In order to assess the changes in protein expression levels which may result from potential interactions between the events, protein levels were compared between the two-event stack and the corresponding single events in different parts of the plant, grown under the same herbicide regimes.

The levels of the 2mEPSPS and PAT proteins in the two-event stack and the corresponding singles were similar in all tissues in both data sets. Levels of HPPD W336 in FG72 × A5547-127 in the Brazil data set were low and could not be quantified, whereas in the single, although still low, they were high enough to be quantifiable, suggesting that there may be a difference of expression between the stack and the single. In the USA data set (Appendix A), levels of HPPD W336 were also low in grain, but could be quantified in the single and the stack. The levels of expression of HPPD W336 in all other tissues were higher than in the grain. The reported expression levels of HPPD W336 in most tissues in the stack were lower than in the single (maximum ratio of twofold), although with overlapping ranges. Such variation in protein expression levels is not unexpected. Therefore, taking into account data from both reports, no evidence for interaction between the events resulting in significant changes of the levels of the newly expressed proteins was identified.

3.3.4. Conclusion

The molecular data establish that the events stacked in soybean FG72 × A5547-127 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins are similar in the two-stack soybean and in the single events. Therefore, there is no indication of an interaction between the events that may affect their integrity and 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 biological level are expected.

3.4. Comparative analyses

3.4.1. Production of material for the comparative assessment and choice of comparator

Application EFSA-GMO-NL-2013-120 presents data on agronomic and phenotypic characteristics and on seed composition of soybean FG72 × A5547-127 derived from field trials performed in the US in 2012 (Table 5). Eight field trial sites were used to generate data both for agronomic and phenotypic characteristics and for seed composition; a ninth site was used only for agronomic and phenotypic characteristics (Table 5). The field trials were conducted in major soybean growing areas of the US, representing regions of diverse agronomic practices and environmental conditions.

To expand the range of possible receiving environments of soybean FG72 × A5547-127, the two stacked events were introgressed into two different genetic backgrounds (soybean MST24 and MST39), adapted to maturity groups 2 and 3, respectively. This is documented by the pedigree, where the production of two different FG72 × A5547-127 lines through three backcrosses is described, namely FG72 × A5547-127 in MST24 genetic background and FG72 × A5547-127 in MST39 genetic background. Given the number of backcrosses, the genetic background of the GM lines is expected to be similar to the one of the respective non-GM soybean variety (MST24 and MST39). Soybean varieties MST24 and MST39 have a genetic background similar to the two different FG72 × A5547-127 lines and were used as comparators in the field trials accordingly (Table 5). The GMO Panel considers that these non-GM soybean varieties are appropriate non-GM comparators.

At each site, only one GM soybean line was tested, i.e. either FG72 × A5547-127 in MST24 or FG72 × A5547-127 in MST39. The appropriate non-GM comparator (soybean MST24 or MST39) was used at each site accordingly.

At each site, the following materials were grown in a randomised complete block design with four replicates: soybean FG72 × A5547-127 untreated (exposed to the conventional herbicides only), soybean FG72 × A5547-127 treated (treated with the intended herbicides, i.e. glyphosate, glufosinate-ammonium and isoxaflutole-based herbicides), the appropriate non-GM comparator (soybean MST24 or MST39) and three non-GM soybean reference varieties. All materials were treated (sprayed) with maintenance pesticides (including conventional herbicides) according to local requirements. Across all the field trial sites, a total of six non-GM soybean reference varieties[10] were used.

[10] The non-GM reference varieties were Stine 2020-0, Stine 2212-0, Stine 2500-2, Stine 3400-2, Stine 3900-2 and Stine 4400-2.

Soybean FG72 × A5547-127 in MST39 was used to test seed germination. The GM line was compared with the single soybean events FG72 and A5547-127 and with the non-GM comparator MST39 (Table 5).

Table 5: Overview of comparative assessment studies with soybean FG72 × A5547-127 provided in application EFSA-GMO-NL-2013-120

Study focus	Study details	Comparators	Commercial non-GM reference varieties
Agronomic and phenotypic characteristics	Field trials, 2012, US (nine locations[(a)])	MST24[(b)] MST39[(c)]	Six
Compositional analysis	Field trials, 2012, US (eight locations[(d)])	MST24 MST39	Six
Agronomic and phenotypic characteristics	Seed germination test	MST39 FG72 A5547-127	None

(a): The field trials were located in two maturity group regions for soybean cultivation. In the field trial sites located in Leonard, MO; York, NE; Carlyle, IL; Stewardson, IL; Richland, IA; Kimballton, IA and Louisville, NE test materials suitable for maturity group 2 regions were selected, while soybean varieties of maturity group 3 were selected for field trial sites located in Kirklin, IN and Fisk, MO.

(b): At sites of maturity group 2, soybean FG72 × A5547-127 in MST24 genetic background was used as GM line, the variety MST24 as non-GM comparator and Stine 2020-0, Stine 2212-0 and Stine 2500-2 as non-GM commercial varieties.

(c): At sites of maturity group 3, soybean FG72 × A5547-127 in MST39 genetic background was used as GM line, the variety MST39 as non-GM comparator and Stine 2400-2, Stine 3900-2 and Stine 4400-2 as non-GM commercial varieties.

(d): For the compositional analysis, the data were collected from the same field trials as for the agronomic and phenotypic characterisation, with the exception of the site Carlyle, IL, for which no compositional data were collected.

3.4.1.1. Statistical analysis of field trials data

The statistical analysis of the agronomic, phenotypic and compositional data from the 2012 field trials followed the recommendations of the GMO Panel (EFSA GMO Panel, 2010b, 2011a). This includes, for each of the two treatments of soybean FG72 × A5547-127, the application of a difference test between the GM soybean and the non-GM comparator. Here and in the rest of this scientific opinion, the term 'non-GM comparator' is used to refer to both genetic backgrounds (soybean MST24 and MST39, Table 5); reference to a specific genetic background is made only if/when necessary. The analysis also includes an equivalence test between the GM soybean and the set of non-GM commercial reference varieties, the results of the equivalence test being categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).[11]

3.4.2. Agronomic and phenotypic analysis

3.4.2.1. Agronomic and phenotypic characteristics tested under field conditions[12]

Twenty-one phenotypic and agronomic characteristics were evaluated on the basis of data collected from the nine field trial sites in the US in 2012 (Table 5).[13]

Seven endpoints[14] were measured as categorical data and therefore not suitable for a parametric analysis: for these, differences between the GM soybean and the non-GM comparator were investigated with the Cochran–Mantel–Haenszel (CMH) test. Other qualitative phenotypic characteristics[15] were not subjected to statistical analysis.

Statistically significant differences were identified between soybean FG72 × A5547-127 (treated and untreated) and the non-GM comparator for the endpoints 'stand count' (early and final) and 'plant

[11] 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).

[12] Dossier: Part II – Section A.3.4; additional information: 19/11/2015 and 12/8/2016.

[13] Emergence, early stand count, plant vigour, plant health at an early developmental stage, flowering date, flower colour, leaf shape, canopy architecture, plant health at a mid-developmental stage, pubescence colour, pod colour, hilum colour, plant height, days to maturity, yield, plant lodging, stand count, pod shattering (at maturity), pod shattering (2 weeks after maturity), growth habit and plant health at a late developmental stage.

[14] Categorical endpoints: plant vigour, plant health (early, mid and late developmental stage), plant lodging and pod shattering (at maturity and 2 weeks after maturity).

[15] Qualitative endpoints: flower colour, leaf shape, canopy architecture, pubescence, pod, hilum colour and growth habit.

height'. For these endpoints, a reduction was observed for the GM soybean FG72 × A5547-127 compared with the non-GM comparator.[16] These endpoints fell within the equivalence limits established by the non-GM reference varieties (equivalence category I). Of the endpoints analysed with CMH test, a statistically significant reduction was identified in soybean FG72 × A5547-127 compared with the non-GM comparator[17] for plant vigour and pod shattering at maturity. For these endpoints, the average values fell within the range of the non-GM reference varieties. However, for the endpoints stand count (early and final) and plant vigour the per-site summary statistics revealed that the observed differences can be mainly attributed to data derived from the sites where soybean FG72 × A5547-127 introgressed into MST39 was tested (maturity group 3 sites), where a reduction of *ca.* 50% was observed. Such reduction was not observed for the single events FG72 and A5547-127 previously assessed by the GMO Panel (EFSA GMO Panel, 2011c, 2015). The GMO Panel requested further information, but from the submitted data,[18] it was not possible to fully characterise the observed differences. The GMO Panel further assessed the potential environmental impacts of such differences in Section 3.6.2.1.

3.4.2.2. Agronomic and phenotypic characteristics tested under controlled conditions[19]

Seed germination of soybean FG72 × A5547-127[20] was compared with that of the non-GM comparator MST39 and of the two parental lines, soybean FG72 and A5547-127, under warm and cold conditions. Eight replicates of 50 seeds for each line were tested for warm germination conditions while four replicates of 50 seeds for each line were tested for cold germination conditions. The warm treatment consisted of exposure to a constant temperature of 25 ± 5°C for 8 days, while the cold treatment consisted of exposure to 10 ± 5°C for 7 days followed by additional exposure to 25 ± 5°C for 8 days. The germination rate of soybean FG72 × A5547-127 under warm and cold conditions did not differ significantly from that of the non-GM comparator and the two parental lines.

3.4.3. Compositional analysis[21]

Soybean seeds harvested from the field trials in the USA in 2012 (Table 5) were analysed for 82 constituents,[22] including the key constituents recommended by the OECD (OECD, 2012). For 18 constituents,[23] more than one-third of the observations were below the limit of quantification. The statistical analysis was applied to the remaining 64 constituents.

[16] Estimated mean values for early stand count (plants per plot): 80 (soybean FG72 × A5547-127 untreated), 75.5 (soybean FG72 × A5547-127 treated) and 88.5 (non-GM comparator). Estimated mean values for final stand count (plants per plot): 72.8 (soybean FG72 × A5547-127 untreated), 73.6 (soybean FG72 × A5547-127 treated) and 84.5 (non-GM comparator). Estimated mean values for plant height (cm): 51.1 (soybean FG72 × A5547-127 untreated), 50.2 (soybean FG72 × A5547-127 treated) and 52.9 (non-GM comparator).

[17] Average rating for plant vigour: 6.9 (soybean FG72 × A5547-127 untreated), 6.9 (soybean FG72 × A5547-127 treated) and 7.4 (non-GM comparator). Average rating for pod shattering at maturity: 8.6 (soybean FG72 × A5547-127 untreated), 8.3 (soybean FG72 × A5547-127 treated) and 8.8 (non-GM comparator). Average rating for pod shattering 2 weeks after maturity: 7.8 (soybean FG72 × A5547-127 treated) and 8.3 (non-GM comparator); for soybean FG72 × A5547-127 (untreated), the endpoint was not significantly different.

[18] Additional information: 12/8/2016.

[19] Dossier: Part II – Section E.3.1.

[20] Generation BC3F8 of FG72 × A5547-127 in MST39 genetic background was used for seed germination studies.

[21] Dossier: Part II – Section A.3.3; additional information: 19/11/2015.

[22] Proximates and fibre (moisture, protein, fat, ash, acid detergent fibre (ADF), neutral detergent fibre (NDF) and carbohydrate), minerals (calcium, potassium, phosphorus, magnesium, sodium and iron), vitamins (vitamins A, B1, B2 and K (total), folic acid and α-, β-, γ-, δ- and total tocopherols), antinutrients (trypsin inhibitors, lectins, phytic acid, stachyose and raffinose), isoflavones (daidzin, genistin, glycitin, daidzein, genistein, glycitein and total isoflavones), 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 (caprylic (C8:0), capric (C10:0), lauric (C12:0), myristic (C14:0), myristoleic (C14:1), pentadecanoic (C15:0), pentadecenoic (C15:1), palmitic (C16:0), palmitoleic (C16:1), heptadecanoic (C17:0), heptadecenoic (C17:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), octadecatetraenoic (18:4), arachidic (C20:0), eicosenoic (C20:1), eicosadienoic (C20:2), eicosatrienoic (C20:3), arachidonic (C20:4), eicosapentaenoic (C20:5), behenic (C22:0), erucic (C22:1), docosapentaenoic (C22:5 N6), docosapentaenoic (C22:5 N3), docosahexaenoic (C22:6) and lignoceric (C24:0)).

[23] Sodium, daidzein, genistein, glycitein and the fatty acids caprylic (C8:0), capric (C10:0), lauric (C12:0), myristoleic (C14:1), pentadecanoic (C15:0), pentadecenoic (C15:1), octadecatretraenoic (C18:4), arachidonic (C20:3), eicosatrienoic (C20:4), eicosapentaenoic (C20:5), erucic (C22:1), docosapentaenoic (C22:5, N6), docosapentaenoic (C22:5, N3) and docosahexaenoic (C22:6).

For three endpoints,[24] equivalence could not be determined because the variation among the non-GM reference varieties was estimated to be zero. No significant differences were identified for these endpoints between soybean FG72 × A5547–127 and the non-GM comparator.

The combination of test of difference and test of equivalence could be applied to the remaining 61 endpoints, with the following results:

- Statistically significant differences between soybean FG72 × A5547-127 (untreated) and the non-GM comparator were identified for 28 endpoints.[25] All the endpoints fell under equivalence category I.
- Statistically significant differences between soybean FG72 × A5547-127 (treated) and the non-GM comparator were identified for 39 endpoints.[26] All the endpoints fell under equivalence category I.

The GMO Panel assessed all the compositional differences between soybean FG72 × A5547-127 and the non-GM comparator. After considering the well-known biological role of the compounds, the outcome of the equivalence test and the magnitude of the changes observed, the GMO Panel did not identify any need for further food/feed safety assessment.

No compositional data on forage was submitted by the applicant. However, soybean forage is not expected to be imported in a significant amount for use as feed.

3.4.4. Conclusion

The GMO Panel concludes that none of the differences identified between soybean FG72 × A5547-127 and the non-GM comparator in seed composition and agronomic and phenotypic characteristics needs further assessment regarding food and feed safety. Although the GMO Panel cannot conclude on forage composition, soybean forage is not expected to be imported in a significant amount for use as feed.

None of the differences identified between soybean FG72 × A5547-127 and the non-GM comparator in agronomic and phenotypic characteristics tested under field conditions needs further assessment for potential environmental impact except for stand count (early and final) and plant vigour (see Section 3.6.2.1). No relevant differences between soybean FG72 × A5547-127 and the non-GM comparator were observed with regard to seed germination tested under controlled conditions.

3.5. Food and feed safety assessment

3.5.1. Effect of processing[27]

3.5.1.1. Processed products

Based on the outcome of the comparative assessment (Section 3.4), processing of soybean FG72 × A5547-127 into food and feed products is not expected to result in products being different from those of commercial non-GM soybean varieties.

3.5.2. Toxicology

3.5.2.1. Toxicological assessment of newly expressed proteins

Three proteins (HPPD W336, 2mEPSPS and PAT) are newly expressed in the two-event stack soybean FG72 × A5547-127 (Section 3.3.1). The GMO Panel has previously assessed these proteins individually in the context of the single events and no safety concerns for humans or animals were identified.

[24] ADF, iron and heptadecenoic acid (C17:1).

[25] Moisture, protein, fat, NDF, vitamin A, vitamin B1, vitamin K (total), α-tocopherol, γ-tocopherol, δ-tocopherol, total tocopherols, phytic acid, daidzin, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, phenylalanine, proline, serine, threonine, valine, myristic acid (C14:0), palmitic acid (C16:0) and lignoceric acid (C24:0).

[26] Moisture, protein, fat, NDF, carbohydrate, potassium, vitamin A, vitamin B1, vitamin B2, vitamin K (total), α-tocopherol, β-tocopherol, γ-tocopherol, total tocopherols, trypsin inhibitors, phytic acid, daidzin, total isoflavones, alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, phenylalanine, proline, serine, threonine, tyrosine, valine, myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), arachidic acid (C20:0) and eicosenoic acid (C20:1).

[27] Dossier: Part II – Section A.3.5.

The three enzymatic proteins catalyse distinct biochemical reactions: PAT acts on the herbicide glufosinate; HPPD W336 and 2mEPSPS act on different substrates in the plant. On the basis of the known biological function of the individual newly expressed proteins (Table 3), there is currently no expectation for possible interactions relevant to the food and feed safety assessment of the two-event stack soybean FG72 × A5547-127.

The GMO Panel concludes that there are no safety concerns for human and animal health related to the newly expressed proteins HPPD W336, 2mEPSPS and PAT in the two-event stack soybean FG72 × A5547-127.

3.5.2.2. Toxicological assessment of components other than newly expressed proteins

None of the differences identified in seed composition between soybean FG72 × A5547-127 and the non-GM comparator (Section 3.4) required further assessment. No further food and feed safety assessment of components other than the newly expressed proteins is therefore required.

3.5.3. Animal studies with the food/feed derived from GM plants

No animal studies with food/feed derived from the two event stack soybean FG72 × A5547-127 were provided by the applicant.

No substantial modifications in the composition of the food and feed derived from the two-event stack soybean and no indication of possible unintended effects or interactions between the events were identified during the comparative assessment (Section 3.4). Therefore, no animal studies on the food and feed derived from soybean FG72 × A5547-127 are required (EFSA GMO Panel, 2011a).

3.5.4. Allergenicity

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

3.5.4.1. Assessment of allergenicity of the newly expressed proteins[28]

The GMO Panel has previously evaluated the safety of the 2mEPSPS, HPPD W336 and PAT proteins, and no concerns on allergenicity were identified in the context of the applications assessed (Table 1). No new information on allergenicity of the newly expressed 2mEPSPS, HPPD W336 and PAT proteins that might change the previous conclusions of the GMO Panel has become available. Based on current knowledge, and as none of the newly expressed proteins showed allergenicity, no reasons for concern regarding the simultaneous presence of these newly expressed proteins in the two-event stack soybean affecting their allergenicity were identified.

For adjuvanticity, there is no information available on the structure or function of the newly expressed 2mEPSPS, HPPD W336 and PAT proteins that would suggest an adjuvant effect of the proteins, individually or in combination, in soybean FG72 × A5547-127 resulting in or increasing an eventual immunoglobulin E (IgE) response to a bystander protein.

3.5.4.2. Assessment of allergenicity of the whole GM plant[29]

Soybean is considered to be a common allergenic food[30] (OECD, 2012). Therefore, any potential change in the endogenous allergenicity of the GM plant when compared with that of the non-GM comparator should be assessed (EFSA GMO Panel, 2011a). Such assessments were performed for the

[28] Dossier: Part II – Sections A.5.1 and A.5.3.
[29] Dossier: Part II – Section A.5.2.
[30] 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.

single events soybean FG72 and soybean A5547-127, and no reasons for concerns on allergenicity were identified by the GMO Panel (Table 1).

To support the safety of soybean FG72 × A5547-127, the applicant performed two-dimensional (2D) electrophoresis of whole protein extracts of soybean FG72 × A5547-127, the non-GM comparator MST39 and three non-GM soybean commercial varieties, followed by Coomassie blue staining. The intensity of specific spots corresponding to five known allergens was analysed by densitometry methods. The outcome of such analyses showed that the genetic modification did not induce a significant increase in the intensity of spots raising concerns for any of the five allergens tested.

In the context of this application, the GMO Panel considers that there is no evidence that the genetic modification might significantly increase the overall allergenicity of soybean FG72 × A5547-127 when compared to that of the non-GM comparator and non-GM soybean commercial varieties.

3.5.5. Nutritional assessment of GM food/feed

The intended trait of the two-event stack soybean FG72 × A5547-127 is herbicide tolerance, with no intention to alter nutritional parameters. The comparison of seed composition between soybean FG72 × A5547-127 and the non-GM comparator did not identify differences that would require a nutritional assessment as regards to food and feed (see Section 3.4). From these data, the nutritional characteristics of soybean FG72 × A5547-127-derived food and feed are not expected to differ from those of food and feed derived from the non-GM comparator.

3.5.6. Conclusion

The newly expressed proteins 2mEPSPS, HPPD W336 and PAT in the two-event stack soybean do not raise safety concerns for human and animal health. No interactions between these proteins relevant for food and feed safety were identified. Similarly, the GMO Panel did not identify indications of safety concerns regarding allergenicity or adjuvanticity related to the presence of the newly expressed proteins in soybean FG72 × A5547-127, or regarding the overall allergenicity of the two-event stack soybean. The nutritional value of food and feed derived from soybean FG72 × A5547-127 is not expected to differ from that of food and feed derived from the non-GM comparator.

3.6. Environmental risk assessment

3.6.1. Evaluation of relevant scientific data

Considering the scope of application EFSA-GMO-NL-2013-120 (which excludes cultivation), the ERA of soybean FG72 × A5547-127 is mainly concerned with: (1) the exposure of bacteria to recombinant DNA in the gastrointestinal tract of animals fed GM material and bacteria present in environments exposed to their faecal material (manure and faeces); and (2) the accidental release into the environment of viable soybean FG72 × A5547-127 seeds during transportation and processing (EFSA GMO Panel, 2010a).

3.6.2. Environmental risk assessment[31]

3.6.2.1. Persistence and invasiveness of the GM plant[32]

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 (Lu, 2005). Cultivated soybean seeds rarely display any dormancy characteristics and can grow as volunteers in the year after cultivation only under certain environmental conditions. If volunteers occur, they do not compete well with the succeeding crop, and can easily be controlled mechanically or chemically (OECD, 2000). The presence of volunteers of *G. max* was occasionally reported in some areas of Italy where soybean is intensively cultivated (Celesti-Grapow et al., 2010). However, soybean seeds usually do not survive during the winter owing to absence of a dormancy phase, herbivory, rotting and germination, or owing to management practices prior to planting the subsequent crop (Owen, 2005). Also, survival of soybean plants outside cultivation areas is limited mainly by a combination of low competitiveness, absence of a dormancy phase, and susceptibility to plant pathogens and cold climatic conditions.

[31] Dossier: Part II – Sections E1, E2 and E3.
[32] Dossier: Part II – Sections E3.1 and additional information: 28/4/2016.

Soybean FG72 × A5547-127 has been developed to confer tolerance to glyphosate-, glufosinate-ammonium- and isoxaflutole (IFT)-based herbicides. The *2mepsps*, *pat* and *hppdPfW336* genes coding for a herbicide tolerance traits can provide a potential agronomic and selective advantage for this GM soybean plant when glyphosate-, glufosinate-ammonium or IFT-based herbicides are applied.

The applicant presented agronomic and phenotypic data on soybean FG72 × A5547-127 gathered from field trials conducted in soybean growing areas in the US (Section 3.4.2). Relevant significant reduction in stand count (early and final) and plant vigour for soybean FG72 × A5547-127 were identified.[33] No further relevant difference for the measured plant characteristics of soybean FG72 × A5547-127 including yield components was observed. Although it was not possible to fully characterise the observed differences, as the general characteristics of soybean FG72 × A5547-127 remain unchanged compared to the non-GM comparator, it is considered very unlikely that soybean FG72 × A5547-127 will differ from conventional soybean varieties in its ability to survive until subsequent seasons, or to establish occasional feral plants under European environmental conditions.

Due to the low survival capacity of soybean, the observed differences are unlikely to change the fitness (e.g. survival, fecundity, competitiveness) or invasiveness characteristics of soybean FG72 × A5547-127 plants. In the case of accidental release into the environment of viable soybean FG72 × A5547-127 seeds during transportation and processing, there are no indications of an increased likelihood of establishment and spread of occasional feral soybean FG72 × A5547-127 plants, unless they are exposed to glyphosate-, glufosinate-ammonium or IFT-based herbicides. However, the possible exposure to these herbicides would not result in different environmental impacts compared to conventional soybean.

The EFSA GMO Panel is not aware of any scientific report of increased survival capacity, including overwintering, of existing GM soybeans varieties (Dorokhov et al., 2004; Owen, 2005; Bagavathiannan and Van Acker, 2008; Lee et al., 2009). Therefore, the GMO Panel is of the opinion that the likelihood of environmental effects of soybean FG72 × A5547-127 in Europe will not be different from that of conventional soybean varieties.

3.6.2.2. Effects of gene transfer[34]

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

1) Plant-to-bacteria gene transfer

The potential for HGT of the recombinant DNA of the single events has already been assessed in previous opinions (see Table 1) and no concern for an unlikely, but theoretically possible, HGT of the recombinant genes to bacteria in the gut of animal fed GM material or other receiving environments was identified. For this application, the plant-to-microorganism gene transfer potential also considered the bioinformatic analyses provided by the applicant.

For event FG72, the bioinformatic analysis revealed two complete insert copies arranged in head-to-tail orientation, both including two regions with sufficient bacterial sequence identity, thus bearing the potential for facilitating homologous recombination: (1) the *hppdPfW336* gene (99% identity over 1,107 bp) and (2) the 3′ *nos* terminator (100% identity over 254 bp). The *hppdPfW336* gene is derived from *P. fluorescens* and homology is found with other bacterial species e.g. *Pseudomonas simiae* and *Pseudomonas azotoformans.* The 3′ *nos* terminator of nopaline is derived from the Ti plasmids of *Agrobacterium tumefaciens* (*Rhizobium radiobacter*). Considering the presence of the two complete insert copies, homologous recombination between *hppdPfW336* genes could theoretically result in the acquisition of genetic elements located between these regions, i.e. the *2mepsps* gene. This gene itself, isolated from *Zea mays*, does not show similarity with known bacterial genomes, and it is expected to be not (or dramatically less) functional in *Pseudomonas fluorescens* or other potential bacterial recipients compared to plants. Homologous recombination between the 3′ *nos* regions could theoretically result in the additional acquisition of the *hppdPfW336* gene on *A. tumefaciens* nopaline-type Ti plasmids and, thus, confer tolerance to *p*-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides (such as isoxaflutole) by reducing the specificity for the herbicide's bioactive constituent. Insertion of the gene in the 3′ *nos* terminator signal could reduce the expression of the nopaline synthase gene and hence the growth of the crown gall tumour. Theoretically, this could provide a selective advantage to bacterial recipients provided that they are naturally susceptible and exposed to

[33] Additional information: 12/8/2016.
[34] Dossier: Part II – Sections E 3.1 and 3.2.

such herbicidal compounds. Such exposure is not expected in the main receiving environment, i.e. the gastrointestinal tract of animals fed with soybean FG72 × A5547-127.

No, or only a very low natural prevalence of *P. fluorescens, P. simiae*, *P. azotoformans* and *A. tumefaciens* in the main receiving environments (gastrointestinal tract) is expected. Considering the generally low exposure of environmental bacteria to HPPD-inhibiting herbicides, the expected natural environmental prevalence of *P. fluorescens* with *hppdPfW336* gene, and the naturally ongoing processes for HGT between bacteria, it is unlikely that double homologous recombination as described above with DNA originating from soybean FG72 × A5547-127 confers an environmental risk.

For event A5547-127, bioinformatics analysis revealed two regions with sufficient bacterial sequence identity in the same orientation, thus bearing potential for facilitating homologous recombination: the 5′ region of the *bla* gene (about 389 bp with 100% identity to the bacterial gene) and the 1,675 bp plasmid sequence corresponding to the pUC backbone vector including the 3′ sequence of the *bla* gene (about 444 bp), the 5′ sequence of the *lacZ* gene and the ColE1 origin of replication. Homologous recombination is possible between the 3′ and 5′ sequences of the *bla* gene with the insertion of the *pat* gene located in between. This homologous recombination could occur with a chromosomally located *bla* gene, leading to insertion of the *pat* gene chromosomally. Alternatively the homologous recombination could occur within the recombinant plant fragment itself leading, due to the presence of the origin of replication, to a self-replicating plasmid containing the *pat* gene and a restored *bla* gene.

Due to its plant codon optimisation, it is expected that the newly acquired *pat* gene construct of A5547-127 would not provide a functional enzyme and therefore no selective advantage to bacterial recipients would be conferred.

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, in the context of the scope of the application EFSA-GMO-NL-2013-120, the unlikely, but theoretically possible, horizontal transfer of recombinant genes from this two-event stack soybean to bacteria does not raise any environmental safety concern.

In line with its previous assessments of FG72 and A5547-127, and considering the new, additional bioinformatic analyses provided by the applicant,[4] the GMO Panel concludes that the horizontal gene transfer from soybean FG72 × A5547-127 to bacteria is highly unlikely, theoretically possible but does not raise a safety concern.

2) Plant-to-plant gene transfer

Considering the scope of the application EFSA-GMO-NL-2013-120 and the biology of soybean, the potential of occasional feral GM soybean plants originating from seed import spills to transfer recombinant DNA to sexually cross-compatible plants is assessed.

The genus *Glycine* is divided into two distinct subgenera: *Glycine* and *Soja*. The subgenus *Glycine* contains 16 perennial wild species, while the cultivated soybean, *Glycine max*, and its wild and semiwild annual relatives, *G. soja* and *G. gracilis*, are classified in the subgenus *Soja* (OECD, 2000). Owing to the low level of genomic similarity among species of the genus *Glycine*, *G. max* can cross with only other members of the *Glycine* subgenus *Soja* under natural conditions (Singh et al., 1987; Hymowitz et al., 1998; Lu, 2005). Hence, the three species of the subgenus *Soja* are capable of cross-pollination and the hybrid seed that is produced can germinate normally and produce plants with fertile pollen and seed (Abe et al., 1999; Nakayama and Yamaguchi, 2002). Since *G. soja* and *G. gracilis* are indigenous to China, Taiwan, Korea, Japan, the far-east region of Russia, Australia, the Philippines and the South Pacific, and since they have not been reported in other parts of the world where the cultivated soybean is grown (Dorokhov et al., 2004; Lu, 2005), the plant-to-plant gene transfer from soybean is restricted to cultivated areas and occasional soybean plants resulting from seed spillage in the EU.

Soybean is an annual, almost completely self-pollinating crop with a percentage of cross-pollination usually below 1% (OECD, 2000; Ray et al., 2003; Lu, 2005; Yoshimura et al., 2006; Abud et al., 2007). Soybean pollen dispersal is limited because the anthers mature in the bud and directly pollinate the stigma of the same flower (OECD, 2000).

However, cross-pollination rates as high as 6.3% have been reported for closely spaced plants (Ray et al., 2003), suggesting the potential for some within-crop gene flow in soybean. These results indicate that natural cross-pollination rates can fluctuate significantly among different soybean varieties under particular environmental conditions, such as favourable climate for pollination and an abundance

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

For plant-to-plant gene transfer to occur, imported soybean FG72 × A5547-127 seeds need to be processed outside the importing ports, transported into regions of soybean production in Europe, spilled during transportation, germinate and develop into plants in the very close vicinity of soybean fields, and there needs to be an overlap of flowering periods and environmental conditions favouring cross-pollination. It must be noted that most soybean FG72 × A5547-127 seeds are processed in the countries of production or in ports of importation. The overall likelihood of cross-pollination between occasional feral GM soybean plants and cultivated soybean is therefore extremely low.

In conclusion, even if cross-pollination would occur, the GMO Panel is of the opinion that the likelihood of environmental effects as a consequence of the spread of genes from occasional feral GM soybean plants in Europe will not differ from that of conventional soybean varieties (see other Sections of 3.6.2.1).

3.6.2.3. Interactions of the GM plant and target organisms[35]

Considering the scope of the application EFSA-GMO-NL-2013-120, and in the absence of target organisms, potential interactions of the GM plant with target organisms were not considered a relevant issue by the GMO Panel.

3.6.2.4. Interactions of the GM plant with non-target organisms[36]

Considering the scope of the application EFSA-GMO-NL-2013-120 and the low level of exposure to the environment, potential interactions of spilled seeds or occasional feral soybean FG72 × A5547-127 plants arising from seed import spills with non-target organisms are not considered a relevant issue by the GMO Panel.

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

Considering the scope of the application EFSA-GMO-NL-2013-120, and the low level of exposure to the environment, potential interactions of occasional feral soybean FG72 × A5547-127 plants arising from seed import spills with the abiotic environment and biogeochemical cycles are not considered a relevant issue by the GMO Panel.

3.6.3. Conclusion

In the case of accidental release into the environment of viable seeds of soybean FG72 × A5547-127 seeds during transportation and processing, there are no indications of an increased likelihood of establishment and spread of occasional feral soybean FG72 × A5547-127 plants, unless these plants are exposed to isoxaflutole-, glyphosate- or glufosinate-ammonium-based herbicides. The GMO Panel is of the opinion that this will not result in different environmental impacts compared to conventional soybean.

Considering the scope of the application EFSA-GMO-NL-2013-120, interactions of soybean FG72 × A5547-127 with the biotic and abiotic environment are not considered to be relevant issues. The HGT from soybean FG72 × A5547-127 to bacteria is highly unlikely, theoretically possible but does not raise a safety concern. Therefore, considering the introduced traits, the outcome of the comparative analysis, the routes of exposure and the limited exposure levels, the GMO Panel concludes that soybean FG72 × A5547-127 would not raise safety concerns in the event of accidental release of viable GM soybean seeds into the environment.

3.7. Post-market monitoring

3.7.1. Post-market monitoring of GM food/feed[38]

No relevant compositional, agronomic and phenotypic changes were identified in soybean FG72 × A5547-127 when compared with its conventional counterpart. Furthermore, the overall intake or exposure is not expected to change because of the introduction of soybean FG72 × A5547-127 into

[35] Dossier: Part II – Section E 3.3.
[36] Dossier: Part II – Section E 3.4.
[37] Dossier: Part II – Section E 3.6.
[38] Dossier: Part II – Section D.

the market. Therefore, the GMO Panel considers that the post-market monitoring of soybean FG72 × A5547-127 is not necessary.

3.7.2. Post-market environmental monitoring[39]

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

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

The PMEM plan proposed by the applicant includes: (1) the description of a monitoring approach involving operators (federations involved in soybean import and processing), reporting to the applicants, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment; (2) a coordinating system established by EuropaBio for the collection of information recorded by the various operators (Lecoq et al., 2007; Windels et al., 2008) and (3) the review of relevant scientific publications retrieved from literature searches. The applicant proposes to submit a PMEM report on an annual basis, and a final report at the end of the consent period. The GMO Panel considers the scope of the PMEM plan provided by the applicant is consistent with the scope of soybean FG72 × A5547-127. As the ERA does not cover cultivation and did not identify potential adverse environmental effects from soybean FG72 × A5547-127, no case-specific monitoring is necessary. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

4. Conclusions

No new data on the single soybean events FG72 and A5547-127 that would lead to a modification of the original conclusions on their safety were identified.

The combination of events FG72 and A5547-127 in the two-event stack soybean FG72 × A5547-127 did not give rise to issues related to molecular, agronomic/phenotypic or seed compositional characteristics regarding food and feed safety. The newly expressed proteins in soybean FG72 × A5547-127 did not raise concerns for human and animal health. The data on seed composition indicate that soybean FG72 × A5547-127 is expected to be as nutritious as the non-GM comparator. Although the GMO Panel cannot conclude on forage composition, soybean forage is not expected to be imported in a significant amount for use as feed. The EFSA GMO Panel considers that there is no reason to expect interactions that could impact on food and feed safety.

The GMO Panel concludes that there is a very low likelihood of environmental effects resulting from the accidental release of viable seeds from soybean FG72 × A5547-127 into the environment. Considering the scope of the application with regard to food and feed uses, interactions with the biotic and abiotic environment are not considered an issue. Risks associated with an unlikely, but theoretically possible, HGT from soybean FG72 × A5547-127 to bacteria have not been identified.

The GMO Panel considers that post-market monitoring of soybean FG72 × A5547-127 products is not necessary. The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of soybean FG72 × A5547-127.

Overall, the GMO Panel concludes that soybean FG72 × A5547-127 is as safe as the non-GM comparator and non-GM soybean reference varieties with respect to potential effects on human and animal health and the environment.

Documentation requested and provided to EFSA

1) Letter from the Competent Authority of the Netherlands received on 10 December 2013 concerning a request for placing on the market of genetically modified soybean FG72 × A5547-127 submitted by Bayer CropScience LP and M.S. Technologies, LLC in accordance with Regulation (EC) No 1829/2003 (application reference EFSA-GMO-NL-2013-120).
2) Acknowledgement letter dated 19 December 2013 from EFSA to the Competent Authority of the Netherlands.

[39] Dossier: Part II – Section E.4.

3) Letter from EFSA to applicant dated 19 December 2013 requesting additional information under completeness check.
4) Letter from applicant to EFSA received on 17 January 2014 providing additional information under completeness check.
5) Letter from EFSA to applicant dated 28 February 2014 requesting additional information under completeness check.
6) Letter from applicant to EFSA received on 31 March 2014 extending the timeline for submission of responses under completeness check.
7) Letter from applicant to EFSA received on 3 June 2014 extending the timeline for submission of responses under completeness check.
8) Letter from applicant to EFSA received on 4 November 2014 extending the timeline for submission of responses under completeness check.
9) Letter from applicant to EFSA received on 2 February 2015 providing additional information under completeness check.
10) Letter from EFSA to applicant dated 23 February 2015 delivering the 'Statement of Validity' of application EFSA-GMO-NL-2013-120 for placing on the market of genetically modified soybean FG72 × A5547-127 submitted by Bayer CropScience LP and M.S. Technologies, LLC in accordance with Regulation (EC) No 1829/2003.
11) Letter from EFSA to applicant dated 24 February 2015 stopping the clock due to single event not finalised (soybean FG72, Application EFSA-GMO-BE-2011-98).
12) Letter from EFSA to applicant dated 30 June 2015 restarting the clock on 25 June 2015 due to finalisation of single event soybean FG72, Application EFSA-GMO-BE-2011-98.
13) Letter from EFSA to applicant dated 07 July 2015 requesting additional information and stopping the clock.
14) Letter from applicant to EFSA received on 20 August 2015 providing additional information.
15) Letter from EFSA to applicant dated 18 September 2015 requesting additional information and maintaining the clock stopped.
16) Letter from applicant to EFSA received on 19 November 2015 providing additional information.
17) Letter from EFSA to applicant dated 29 February 2016 requesting additional information and maintaining the clock stopped.
18) Letter from EFSA to applicant dated 23 March 2016 requesting additional information and maintaining the clock stopped.
19) Letter from applicant to EFSA received on 7 April 2016 providing additional information.
20) Letter from applicant to EFSA received on 28 April 2016 providing additional information.
21) Email from EFSA to applicant, dated 29 April 2016, re-starting the clock from 28 April 2016.
22) Letter from EFSA to applicant dated 15 June 2016 requesting additional information and stopping the clock.
23) Letter from EFSA to applicant dated 28 June 2016 requesting additional information and maintaining the clock stopped.
24) Letter from applicant to EFSA received on 12 August 2016 providing additional information.
25) Letter from applicant to EFSA received on 12 August 2016 providing additional information.
26) Email from EFSA to applicant, dated 16 August 2016, re-starting the clock from 12 August 2016.
27) Letter from EFSA to applicant dated 27 September 2016 requesting additional information and stopping the clock.
28) Letter from applicant to EFSA received on 8 November 2016 providing additional information.
29) Email from EFSA to applicant, dated 9 November 2016, re-starting the clock from 8 November 2016.
30) Letter from EFSA to applicant dated 5 December 2016 requesting additional information and stopping the clock.
31) Letter from applicant to EFSA received on 30 January 2017 providing additional information.
32) Email from EFSA to applicant, dated 30 January 2017, re-starting the clock.

References

Abe J, Hasegawa A, Fukushi H, Mikami T, Ohara M and Shimamoto Y, 1999. Introgression between wild and cultivated soybeans of Japan revealed by RFLP analysis for chloroplast DNAs. Economic Botany, 53, 285–291.

Abud S, de Souza PIM, Vianna GR, Leonardecz E, Moreira CT, Faleiro FG, Júnior JN, Monteiro PMFO, Rech EL and Aragão FJL, 2007. Gene flow from transgenic to nontransgenic soybean plants in the Cerrado region of Brazil. Genetics and Molecular Research, 6, 445–452.

Ahrent DK and Caviness CE, 1994. Natural cross-pollination of 12 soybean cultivars in Arkansas. Crop Science, 34, 376–378.

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

Boudec P, Rodgers M, Dumas F, Sailland A and Bourdon H, 2001. Mutated hydroxyphenylpyruvate dioxygenase, DNA sequence and isolation of plants which contain such a gene and which are tolerant to herbicides. United States Patent No 6245968 B1.

Celesti-Grapow L, Pretto F, Carli E and Blasi C (eds.), 2010. *Flora vascolare alloctona e invasiva delle regioni d'Italia*. Casa Editrice Universita La Sapienza, Rome. 208 pp.

Codex Alimentarius, 2009. *Foods Derived from Modern Biotechnology*. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Rome. ftp://ftp.fao.org/docrep/fao/011/a1554e/a1554e00.pdf

Dorokhov D, Ignatov A, Deineko E, Serjapin A, Ala A and Skryabin K, 2004. Potential for gene flow from herbicide-resistant GM soybeans to wild soya in the Russian Far East. In: den Nijs HCM, Bartsch D, Sweet J (eds.)., *Introgression from Genetically Modified Plants into Wild Relatives*, CAB International. Amsterdam, the Netherlands. pp. 151–161.

EFSA GMO Panel (Panel on Genetically Modified Organisms), 2010a. Guidance on the environmental risk assessment of genetically modified plants. EFSA Journal 2010;8(11):1879. 111 pp. doi:10.2903/j.efsa.2010.1879

EFSA GMO Panel (Panel on Genetically Modified Organisms), 2010b. Statistical considerations for the safety evaluation of GMOs. EFSA Journal 2010;8(1):1250, 59 pp. doi:10.2903/j.efsa.2010.1250

EFSA GMO Panel (Panel on Genetically Modified Organisms), 2011a. Guidance for risk assessment of food and feed from genetically modified plants. EFSA Journal 2011a;9(5):2150, 37 pp. doi:10.2903/j.efsa.2011.2150

EFSA GMO Panel (Panel on Genetically Modified Organisms), 2011b. Guidance on the post-market environmental monitoring (PMEM) of genetically modified plants. EFSA Journal 2011;9(8):2316, 40 pp. doi:10.2903/j.efsa.2011.2316

EFSA GMO Panel (Panel on Genetically Modified Organisms), 2011c. Scientific Opinion on application (EFSA-GMO-NL-2008-52) for the placing on the market of herbicide tolerant genetically modified soybean A5547-127 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience. EFSA Journal 2011; 9(5):2147, 27 pp. doi:10.2903/j.efsa.2011.2147

EFSA GMO Panel (Panel on Genetically Modified Organisms), 2015. Scientific Opinion on an application (EFSA-GMO-BE-2011-98) for the placing on the market of herbicide-tolerant genetically modified soybean FG72 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience. EFSA Journal 2015;13(7):4167, 29 pp. doi:10.2903/j.efsa.2015.4167

Gumisiriza G and Rubaihayo PR, 1978. Factors that influence outcrossing in soybean. Zeitschrift für Acker- und Pflanzenbau/Journal of Agronomy and Crop Science, 147, 129–133.

Herrmann KM, 1995. The shikimate pathway: early steps in the biosynthesis of aromatic compounds. The Plant Cell, 7, 907–919.

Hymowitz T, Singh RJ and Kollipara KP, 1998. The genomes of the *Glycine*. Plant Breeding Reviews, 16, 289–317.

Kikuchi A, Murata K, Tabuchi K and Sakai S, 1993. Inheritance of seed embryo color and investigation of degree of natural cross-pollination in soybeans. Breeding Science, 43, 112.

Lebrun M, Leroux B and Sailland A, 1997. Chimeric gene for the transformation of plants. United States Patent No 5633448.

Lecoq E, Holt K, Janssens J, Legris G, Pleysier A, Tinland B and Wandelt C, 2007. General surveillance: roles and responsibilities, the industry view. Journal of Consumer Protection and Food Safety, 2(S1), 25–28.

Lee B, Kim C-G, Park J-Y, Woong Park K, Kim H-J, Yi H, Jeong S-C, Kee Yoon W and Mook Kim H, 2009. Monitoring the occurrence of genetically modified soybean and maize in cultivated fields along the transportation routes of the Incheon Port in South Korea. Food Control, 20, 250–254.

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

Nakayama Y and Yamaguchi H, 2002. Natural hybridization in wild soybean (*Glycine max* ssp. *soja*) by pollen flow from cultivated soybean (*Glycine max* ssp. *max*) in a designed population. Weed Biology and Management, 2, 25–30.

OECD (Organisation for Economic Co-operation and Development), 2000. Consensus document on the biology of *Glycine max* (L.) *merr.* (soybean). Series on Harmonization of Regulatory Oversight in Biotechnology ENV/JM/MONO(2000)9, No. 15, pp. 1–20.

OECD (Organisation for Economic Co-operation and Development), 2012. Revised consensus document on compositional considerations for new varieties of soybean [*Glycine max* (L.) *merr.*]: Key food and feed nutrients, anti-nutrients, toxicants and allergens.
Owen MDK, 2005. Maize and soybeans – Controllable volunteerism without ferality? In: Gressel J (ed.). *Crop Ferality and Volunteerism*, OECD + Taylor & Francis, Boca Raton, FL. pp. 149–165.
Ray JD, Kilen TC, Abel CA and Paris RL, 2003. Soybean natural cross-pollination rates under field conditions. Environmental Biosafety Research, 2, 133–138.
Singh RJ, Kollipara KP and Hymowitz T, 1987. Intersubgeneric hybridization of soybeans with a wild perennial species, Glycine clandestina Wendl. Theoretical and Applied Genetics, 74, 391–396.
Strauch E, Arnold W, Alijah R, Wohlleben W, Puhler A and Eckes P. 1993. In Planzen wirksames Resistenzgen gegen Phosphinothricin und seine Verwendung. European patent 0275957 B1.
Windels P, Alcalde E, Lecoq E, Legris G, Pleysier A, Tinland B and Wandelt C, 2008. General surveillance for import and processing: the EuropaBio approach. Journal of Consumer Protection and Food Safety, 3(S2), 14–16.
Yoshimura Y, Matsuo K and Yasuda K, 2006. Gene flow from GM glyphosate-tolerant to conventional soybeans under field conditions in Japan. Environmental Biosafety Research, 5, 169–173.

Abbreviations

ADF	acid detergent fibre
bp	base pair
CaMV	cauliflower mosaic virus
CMH	Cochran–Mantel–Haenszel
EFSA GMO Panel	EFSA Panel on Genetically Modified Organisms
ELISA	enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	environmental risk assessment
GM	genetically modified
GMO	genetically modified organism
HGT	horizontal gene transfer
HPPD	4-hydroxyl phenyl-pyruvate-dioxygenase
HR	homologous recombination
IFT	isoxaflutole
IgE	immunoglobulin E
NDF	neutral detergent fibre
nos	nopaline synthase
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PAT	phosphinothricin acetyl-transferase
PMEM	post-market environmental monitoring
UTR	untranslated region

Appendix A – Protein expression data

Table A.1: Means, standard deviation and ranges of protein levels (μg/g dry weight) from soybean FG72 × A5547-127, FG72 and A5547-127, not treated, from field trials performed in USA in 2014

Growth stage	Sample matrix	Entry ID	HPPD W336		2mEPSPS		PAT	
			Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
V5–V6	Leaf	Single[(a)]	30.82 ± 15.32	15.01–66.32	1,842.45 ± 410.96	1,123.29–2,526.73	117.65 ± 29.25	80.53–186.84
		Stack	29.91 ± 13.70	13.67–58.99	2,012.78 ± 266.98	1,659.46–2,410.96	114.26 ± 11.12	98.40–133.87
	Root	Single	16.05 ± 7.33	6.46–32.52	257.05 ± 66.30	176.75–365.66	87.10 ± 24.46	56.67–136.09
		Stack	12.64 ± 3.79	5.50–19.31	234.34 ± 43.64	158.09–309.63	101.98 ± 24.11	66.83–145.64
R1–R2	Flowers	Single	67.07 ± 16.46	36.13–95.42	811.37 ± 272.93	204.84–1,086.69	97.31 ± 22.34	55.06–130.01
		Stack	39.33 ± 7.17	28.85–52.49	642.95 ± 113.90	452.99–874.73	88.41 ± 17.40	61.61–117.03
R3	Leaf	Single	44.90 ± 15.56	23.71–79.67	1,676.49 ± 615.29	864.53–2,828.39	89.34 ± 16.25	65.86–118.81
		Stack	27.78 ± 5.11	18.95–36.61	1,421.19 ± 214.99	1,080.35–1,681.25	82.21 ± 25.59	30.01–112.67
	Root	Single	15.72 ± 11.19	4.48–45.39	239.38 ± 69.49	172.00–364.55	86.44 ± 37.26	50.96–173.95
		Stack	8.02 ± 5.23	< LLOQ–20.58	189.58 ± 55.67	106.31–312.26	71.56 ± 21.07	39.96–105.16
	Forage	Single	105.29 ± 30.85	64.71–160.08	893.06 ± 160.95	613.14–1,196.56	93.54 ± 15.31	63.18–113.70
		Stack	78.21 ± 20.96	33.39–113.46	866.02 ± 219.02	519.78–1,277.72	93.03 ± 18.98	68.04–139.12
Maturity	Grain	Single	2.15 ± 0.51	1.41–3.07	267.65 ± 25.35	233.16–306.60	17.22 ± 1.71	14.17–19.55
		Stack	1.26 ± 0.25	0.89–1.60	245.21 ± 31.61	184.59–282.07	18.18 ± 3.10	13.35–23.28

(a): 'Single' refers to FG72 for HPPD W336 and 2mEPSPS, and to A5547-127 for PAT.