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SCIENTIFIC OPINION

Scientific Opinion on application (EFSA-GMO-NL-2009-64) for the placing on the market of herbicide-tolerant genetically modified soybean BPS-CV127-9 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from BASF Plant Science[1]

EFSA Panel on Genetically Modified Organisms (GMO)[2,3]

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ABSTRACT

Soybean BPS-CV127-9 contains a single insertion locus of the *csr1-2* gene. Stability of the genetic modification was demonstrated. The expression of the acetohydroxyacid synthase large sub-unit from *Arabidopsis thaliana*, conferring tolerance to imidazolinone herbicides, was sufficiently analysed. Bioinformatic analyses did not raise safety issues. No differences were identified in the seed composition that would require further assessment with regard to safety. Regarding agronomic and phenotypic characteristics, a difference in seed weight was identified; however, this difference does not affect the overall safety of this soybean. Although the EFSA GMO Panel cannot conclude on its forage composition, soybean forage is not expected to be imported in significant amount for feed uses. Safety assessment identified no concerns regarding the potential toxicity and allergenicity of the newly expressed proteins or soybean BPS-CV127-9. Compositional data indicating that soybean BPS-CV127-9 is as nutritious as non-GM soybean varieties were supported by the outcome of a chicken feeding study. There are no indications of an increased likelihood of spread and establishment of feral soybean BPS-CV127-9 plants, unless they are exposed to imidazolinone-containing herbicides. Risks associated with an unlikely, but theoretically possible, horizontal transfer of recombinant genes from soybean BPS-CV127-9 to bacteria have not been identified. Considering the scope of this application, interactions with the biotic and abiotic environment are not considered to be a relevant issue. The post-market environmental monitoring plan and reporting intervals are in line with the scope of this application. In conclusion, the EFSA GMO Panel considers that the information available for soybean BPS-CV127-9 addresses scientific comments raised by Member States and that the soybean BPS-CV127-9, as described in this application, is as safe and nutritious as its conventional counterpart and commercial soybean varieties with respect to potential effects on human and animal health and the environment in the context of its intended uses.

[1] On request from the Competent Authority of the Netherlands for an application (EFSA-GMO-NL-2009-64) submitted by BASF Plant Science, Question No EFSA-Q-2009-00360, adopted on 5 December 2013.

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[3] Acknowledgement: The Panel wishes to thank the members of the Working Groups on Molecular Characterisation, Food and Feed Risk Assessment and Environment Risk Assessment for the preparatory work on this scientific opinion; and EFSA staff: Jaime Aguilera, Yann Devos and Yi Liu for the support provided to this scientific opinion.

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KEY WORDS

GMO, soybean (*Glycine max*), BPS-CV127-9, herbicide tolerance, AtAHAS, Regulation (EC) No 1829/2003

SUMMARY

Following the submission of an application (EFSA-GMO-NL-2009-64) under Regulation (EC) No 1829/2003 from BASF Plant Science, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) was asked to deliver a scientific opinion on the safety of herbicide-tolerant genetically modified (GM) soybean BPS-CV127-9 (Unique Identifier BPS-CV127-9). The scope of the application EFSA-GMO-NL-2009-64 is for import, processing and food and feed uses of soybean BPS-CV127-9 within the European Union (EU) in the same way as any non-GM soybean, but excludes cultivation in the EU.

The EFSA GMO Panel evaluated soybean BPS-CV127-9 with reference to the scope and appropriate principles described in its guidance documents for the risk assessment of GM plants and derived food and feed and the post-market environmental monitoring of GM plants. 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 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. Evaluation of environmental impacts and of the post-market environmental monitoring plan was also undertaken.

Soybean (*Glycine max* (L.) Merr.) event BPS-CV127-9 expresses a mutant acetohydroxyacid synthase large sub-unit of *Arabidopsis thaliana* (L.) Heynh., referred to as AtAHAS, conferring tolerance to the imidazolinone class of herbicides. The wild-type, non-mutated AHAS enzyme is inhibited by the herbicide, which leads to a deficiency in branched-chain amino acids in the absence of the herbicide-tolerant enzyme variant. The molecular characterisation data provided by the applicant establish that the genetically modified soybean BPS-CV127-9 contains a single insertion. Rearrangements of the plant genome were shown at the insertion site; however, bioinformatic analyses and genetic stability studies did not raise any safety issues. The levels of the AtAHAS protein in soybean BPS-CV127-9 have been sufficiently analysed.

No differences were identified in the compositional data of seeds obtained from soybean BPS-CV127-9 that would require further assessment with regard to safety by the GMO Panel. Regarding the agronomic and phenotypic characteristics of soybean BPS-CV127-9, a difference in seed weight was identified. However, this difference does not affect the overall safety of the soybean BPS-CV127-9. 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.

The AtAHAS protein is degraded by proteolytic enzymes, and bioinformatics-supported studies did not identify similarity to known toxic and allergenic proteins. The Panel concluded that soybean BPS-CV127-9 is as safe as its conventional counterpart and that the overall allergenicity of the whole plant is not changed. A feeding study with broiler chickens confirmed that the nutritional properties of soybean BPS-CV127-9 seeds are not different from those of its conventional counterpart and commercial non-GM soybean varieties. In conclusion, the EFSA GMO Panel is of the opinion that soybean BPS-CV127-9 is as safe and nutritious as non-GM soybean varieties, and concludes that soybean BPS-CV127-9 and derived products are unlikely to have adverse effects on human and animal health, in the context of its intended uses.

The application EFSA-GMO-NL-2009-64 covers the import, processing and food and feed uses of soybean BPS-CV127-9 and excludes cultivation. Therefore, there is no requirement for scientific information on possible environmental effects associated with the cultivation of soybean BPS-CV127-9 in Europe. There are no indications of an increased likelihood of spread and establishment of feral soybean BPS-CV127-9 plants in the event of the accidental release into the environment of viable soybean BPS-CV127-9 seeds during transport and/or processing, unless these plants are exposed to imidazolinone-containing herbicides. Given the scope of this application, only a low-level exposure of environmental bacteria, including those in the gastrointestinal tract, to

recombinant DNA from soybean BPS-CV127-9 is expected. The unlikely, but theoretically possible, transfer of the recombinant genes from soybean BPS-CV127-9 to bacteria does not raise concerns owing to the lack of a selective advantage which would be provided to the recipients in the receiving environments. Considering the scope of this application, the risk to non-target organisms is extremely low owing to the rare occurrence of feral soybean plants and the low levels of exposure through other routes. Interactions with the biotic and abiotic environment are therefore not considered a relevant issue. The scope of the post-market environmental monitoring plan provided by the applicant is in line with the scope of this application. Furthermore, the EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the post-market environmental monitoring plan.

In delivering its scientific opinion, the EFSA GMO Panel considered the application EFSA-GMO-NL-2009-64, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications. In conclusion, the EFSA GMO Panel considers that the soybean BPS-CV127-9, as described in this application, is as safe as its conventional counterpart and commercial soybean varieties with respect to potential effects on human and animal health and the environment in the context of its intended uses.

TABLE OF CONTENTS

BACKGROUND

On 15 January 2009, the European Food Safety Authority received from the Competent Authority of the Netherlands an application (Reference EFSA-GMO-NL-2009-64) for authorisation of GM soybean BPS-CV127-9 (Unique Identifier BPS-CV127-9), submitted by BASF Plant Science within the framework of Regulation (EC) No 1829/2003 on GM food and feed.[4]

After receiving the application EFSA-GMO-NL-2009-64 and in accordance with Articles 5(2)(b) and 17(2)(b) of the Regulation (EC) No 1829/2003, EFSA informed the Member States and the European Commission, and made the summary of the application publicly available on the EFSA website.[5] EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of the Regulation (EC) No 1829/2003. On 26 May 2009 and 26 June 2009, EFSA received additional information requested under completeness check (requested on 24 February 2009 and 22 June 2009, respectively). On 13 July 2009, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to the Member States and the European Commission, and consulted nominated risk assessment bodies of the Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC[6] following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Member States had three months after the date of receipt of the valid application (until 13 October 2009) within which to make their opinion known.

The EFSA GMO Panel carried out an evaluation of the scientific risk assessment of the GM soybean BPS-CV127-9 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 2006a, 2011a) and on the post-market environmental monitoring (PMEM) of GM plants (EFSA, 2006b, 2011b). Furthermore, the EFSA GMO Panel also took into consideration the scientific comments of Member States, the additional information provided by the applicant and the relevant scientific publications.

On 21 September 2009, 18 February 2011, 2 and 24 August 2011, 17 February 2012, 13 June 2012, 19 February 2013, 27 March 2013, 11 April 2013 and 18 November 2013, the EFSA GMO Panel requested additional information from the applicant. The applicant provided the requested information on 26 November 2010, 26 May 2011, 3 November 2011, 25 July 2012, 9 August 2012, 26 March 2013, 16 April 2013, 13 May 2013 and 26 November 2013, respectively. The applicant also spontaneously provided additional information on 13 November 2012, 4 February 2013 and 26 March 2013. After evaluation of the full data package, the EFSA GMO Panel finalised its risk assessment of soybean BPS-CV127-9.

In giving its scientific opinion on soybean BPS-CV127-9 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 six months from the acknowledgement of the valid application. As additional information was requested by the EFSA GMO Panel, the time limit of six months was extended accordingly, in line with Articles 6(1), 6(2), 18(1), and 18(2) of Regulation (EC) No 1829/2003.

[4] 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.

[5] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2009-00360

[6] 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, 12.03.2001, p. 1–38.

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).

TERMS OF REFERENCE

The EFSA GMO Panel was requested to carry out a scientific assessment of soybean BPS-CV127-9 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 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.

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.

ASSESSMENT

1. Introduction

The genetically modified (GM) soybean BPS-CV127-9 (Unique Identifier BPS-CV127-9) was assessed with respect to its scope, taking account of the appropriate principles described in the applicable guidance documents (EFSA, 2006a, b) and, whenever possible, also the current guidance documents (EFSA, 2011a, b). The risk assessment presented here is based on the information provided in the application relating to soybean BPS-CV127-9 submitted in the EU, scientific comments raised by the Member States and relevant scientific publications.

The scope of application EFSA-GMO-NL-2009-64 is for import, processing and food and feed use of soybean BPS-CV127-9 within the EU. Thus, soybean BPS-CV127-9 will be imported into the EU for food or feed uses, as any commercial soybean variety. Soybeans are grown primarily for meal, and oil is a secondary product. Soybean meal is used predominantly as a source of protein in feed rations for livestock, including poultry, swine, and dairy and beef cattle (OECD, 2001). Other components of the soybean plant, including the forage, are also fed, to a limited extent, to animals, primarily to cattle. The major food uses for soybean include the production of soybean oil that is utilised in margarines, shortenings, salad and cooking oils. Heat treatment allows use of soybeans to produce soy sprouts, roasted soybeans and the traditional soy foods, for example miso, soy milk, soy sauce and tofu. The soybean protein fractions (soybean isolate and concentrate) are derived from processing of non-toasted soybean flakes and are used in the production of infant formula and various other food products as a source of amino acids. There is only limited food and animal feed use for unprocessed soybeans.[7]

Soybean (*Glycine max* (L). Merr.) event BPS-CV127-9 expresses a mutant acetohydroxyacid synthase large sub-unit of *Arabidopsis thaliana* (L.) Heynh. The mutant *ahasl* allele (S653N, referred to as *csr1-2* in the literature) confers tolerance to the imidazolinone class of herbicides. The genetic modification in soybean BPS-CV127-9 is intended to improve agronomic performance only and it is not intended to influence the nutritional properties, processing characteristics and overall use of soybean as a crop.

2. Issues raised by the Member States

The comments raised by the Member States are addressed in Annex G of the EFSA overall opinion[8] and were taken into consideration during the evaluation of the risk assessment.

3. Molecular characterisation

3.1. Evaluation of relevant scientific data

3.1.1. Transformation process and vector constructs

Embryonic axis tissue derived from the apical meristem of soybean seed of the commercial variety Conquista was transformed by particle bombardment with a purified, linear DNA fragment derived from plasmid pAC321[9] by digestion with *Pvu*II restriction endonuclease. The resulting *Pvu*II fragment contained the *ahasl* gene, which is a natural mutant allele (S653N) of the gene *ahasl* from *A. thaliana* that encodes acetohydroxyacid synthase (AHAS) large sub-unit. The AHAS enzyme catalyses the first step in the biosynthesis of branched-chain amino acids, and the enzyme encoded by the *ahasl* mutant allele retains the normal catalytic activity while preventing the binding of imidazolinone herbicides. The *ahasl* gene served two purposes in the transformed plant: besides being the target gene, it was used as the selectable marker gene which allowed the transformed cells to grow in the presence of

[7] Dossier: Part I—Section B9.
[8] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2010-00972
[9] Dossier: Part I—Sections C1, C2 and Annexes 1, 2.

imazapyr herbicide. Both the 5′ untranslated region containing the putative promoter and the 3′ untranslated region of the *ahasl* gene cassette were native to the *ahasl* gene of *A. thaliana*.

Besides this ca. 2.6 kb *ahasl* gene cassette, the ca. 6.2 kb gel-purified linear *Pvu*II fragment contained short segments of plasmid DNA at both ends of the fragment. In addition, this *Pvu*II fragment contained regions of the *Arabidopsis* genome flanking the *ahasl* coding sequence: segments of unannotated genome and the gene *AtSEC61γ* (locus *At3g48570*) that encodes the protein transport protein Sec61 sub-unit gamma-3 of a heterotrimeric complex necessary for protein translocation in the endoplasmic reticulum. The *Arabidopsis* DNA outside the *ahasl* cassette is not needed to achieve the desired trait, i.e. herbicide tolerance.

3.1.2. Transgene constructs in the genetically modified plant

The DNA sequences inserted in the BPS-CV127-9 event were characterised by Southern analysis and by polymerase chain reaction (PCR) amplification of both the insert and the flanking regions.[10]

The number of copies of the *Pvu*II fragment was determined from F8 generation by Southern analysis[11] using a combination of three restriction enzymes and three probes covering the entire *Pvu*II fragment. The probes for 5′ and 3′ untranslated regions gave only single hybridisation signals, whereas an additional weak hybridisation signal was observed with the *ahasl* probe. Sequence determination of the entire 4758 bp insert indicated that the additional signal was the result of a duplication of a 376 bp fragment of the *ahasl* gene at the 3′ flanking sequence junction of the BPS-CV127-9 event. Overall, the data demonstrate the insertion of DNA into a single locus.

Sequence analysis indicated that the *ahasl* coding sequence in the soybean event BPS-CV127-9 (referred to as *csr1-2*) differs from the *Arabidopsis* donor sequence by a single nucleotide that results in one amino acid replacement (arginine to lysine at position 272).[11] This conservative amino acid substitution has no impact on the enzymatic activity of the protein or on the tolerance to the herbicide. It is unclear when the mutation occurred, as it may have been already present in the linear DNA molecule that integrated in the event BPS-CV127-9 genome. Sequence comparison revealed that the mutation occurred before the T4 generation and that it has been maintained for the subsequent eight generations. Two additional point mutations are located downstream of the 3′ untranslated region of *ahasl*. The unannotated *Arabidopsis* genomic DNA was not inserted except for 17 bp. The *Arabidopsis AtSEC61γ* gene was inserted together with the 17 bp of the unannotated sequence and 62 bp of its 5′ leader sequence upstream of the start codon (79 bp in total).

The absence of elements derived from the backbone of the plasmid pAC321 in BPS-CV127-9 event has been confirmed by Southern analysis.[11]

The ca. 10 kb DNA sequenced covered 1.3 kb of the 5′ flanking region and 4.5 kb of the 3′ flanking region of the insert. BLASTN analysis of the flanking regions using the latest release of genomic sequence from the Soybean Genome Sequencing Project (version v.3.1.1; http://www.phytozome.net/soybean) showed that these regions are native to the soybean nuclear genome[11] and that they are not contiguous when compared with the published Williams 82 landrace genome sequence (Schmultz et al., 2010). Of the 5′ flanking sequence (1.3 kb) analysed, the “distal” (further away from the insert) 1170 bp showed 99 % identity with a sequence in soybean chromosome 2, and the “proximal” (next to the insert) 141 bp were identical to a sequence in chromosome 18. Of the 3′ flanking sequence (4.5 kb), the proximal 3923 bp showed high identity (98 %) with a sequence in soybean chromosome 2, and the distal 664 bp corresponded to a sequence in chromosome 2. This analysis and the unsuccessful attempts to amplify the pre-insertion locus in the non-transgenic soybean parental variety Conquista with PCR suggest that rearrangement of the flanking genomic regions occurred during the transformation of BPS-CV127-9 soybean. It should be noted, however, that the

[10] Dossier: Part I—Section D2 and Annex 1.
[11] Additional information: 26/11/2010 (Appendix 1), 26/03/2013 (Appendix 1).

comparison was made between Williams 82 landrace and the soybean event BPS-CV127-9 developed from the commercial variety Conquista, and the genomes of the two non-GM soybeans are not expected to be identical.

Bioinformatic analysis indicated that the 5′ flanking sequence contains a region that showed homology to predicted exon and introns of the *Glyma02g36330* annotated gene. This led to the conclusion that disruption of an endogenous gene may have occurred in BPS-CV127-9 as a result of the insertion and/or chromosomal rearrangements in the proximity of the insertion site.[12] The annotated gene has no known function.

In order to assess whether the open reading frames (ORFs) present within the insert and spanning the junction sites raise any safety issues, their putative translation products were compared to databases for similarities to known allergens and toxins using suitable algorithms. No significant similarities were found.[13]

3.1.3. Information on the expression of the insert

The expression levels of AtAHAS were measured by enzyme-linked immunosorbent assay (ELISA) in tissues of the soybean BPS-CV127-9 event and the isogenic control grown in multilocation replicated field trials in two different growing seasons in Brazil (13 locations in total). All plots of BPS-CV127-9 were sprayed with an imidazolinone herbicide, and all plots of the conventional counterpart with conventional herbicide. The level of AtAHAS in fully matured grain was barely detectable, being at or below the limit of quantification (15 ng AtAHAS/g fresh weight) both in BPS-CV127-9 soybean and the conventional counterpart.[14]

In addition to the intended *csr1-2* gene, the insert contains the *AtSEC61*γ sub-unit gene locus from *Arabidopsis* containing 79 bp upstream of the start codon. Reverse transcription (RT)-PCR analysis of RNA extracted from the F7 generation was performed and indicated that the gene is weakly transcribed in the leaves of BPS-CV127-9 event, whereas the endogenous *SEC61*γ gene of soybean is strongly expressed in young soybean leaves.[11] No AtSEC61γ sub-unit protein was detectable in Western analysis of a microsomal membrane protein fraction from leaves and fully matured grains.[15] Because of the very low expression levels found in leaves and grains with no detectable proteins in those tissues, significant expression in other tissues is not expected, although no experimental data were provided.

3.1.4. Inheritance and stability of inserted DNA

The integration of the insert in the nuclear genome was confirmed by Southern analysis, PCR and DNA sequence analysis.[16] Stability of the inserted DNA was studied by Southern analysis from four generations. The insert was stable and followed the Mendelian inheritance pattern of a single locus. Phenotypic stability was indicated by analysing the AtAHAS protein from two generations grown in two different growing seasons.

3.2. Conclusion

The molecular characterisation data provided by the applicant establish that the genetically modified soybean BPS-CV127-9 contains a single insertion. Rearrangements of the plant genome were shown at the insertion site; however, bioinformatic analysis of the insert and the 5′ and 3′ flanking regions did not reveal disruption of known genes or creation of ORFs that would raise a safety issue. The stability of the inserted DNA was confirmed over several generations and a Mendelian inheritance pattern was

[12] Additional information: 26/11/2010 (Appendix 1), 26/03/2013 (Appendix 1).
[13] Dossier: Part I—Annex 3; Additional information: 26/11/2010 (Appendix 2), 26/03/2013 (Appendix 2).
[14] Dossier: Part I—Section D3 and Annexes 4, 5.
[15] Dossier: Part I—Section D3 and Annex 6.
[16] Dossier: Part I—Section D5 and Annex 1.

demonstrated. The EFSA GMO Panel concludes that the molecular characterisation of soybean BPS-CV127-9 does not raise any safety issues. The potential impacts of the AtAHAS protein levels, quantified in field trials carried out in Brazil, are assessed in the Sections on the food/feed safety assessment and environmental risk assessment (Sections 5 and 6).

4. Comparative analysis

4.1. Evaluation of relevant scientific data

The applicant performed the comparative assessment using the most recent statistical methodology recommended by the EFSA GMO Panel (EFSA, 2010b, 2011a). This recommends the simultaneous application of a test of difference to determine whether the GM plant is different from its conventional counterpart, and a test of equivalence to determine whether the GM plant falls within natural variation estimated from the non-GM soybean reference varieties included in the study. As described in EFSA (2011a), the result of the equivalence test is categorised into four possible outcomes to facilitate the drawing of conclusions with respect to the presence or absence of equivalence. These four categories are category I, indicating full equivalence; category II, indicating that equivalence is more likely than non-equivalence; category III, indicating that non-equivalence is more likely than equivalence; and category IV, indicating non-equivalence.

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

Field trials for the comparative assessment of soybean BPS-CV127-9 were conducted in soybean growing areas in Brazil during the growing seasons 2006/2007 (seven locations),[17] 2007 (six locations),[18] 2007/2008 (seven locations)[19] and 2009/2010 (seven locations).[20] An additional field trial was performed in a soybean growing area in the USA during the growing season 2011 (one location).[21]

In the 2006/2007, 2007 and 2007/2008 field trials, a negative segregant was used as the only comparator (Table 1). Potential unintended differences in the GM plant owing to the genetic modification cannot be discounted using a negative segregant as the *only* comparator (EFSA, 2011a). Therefore, the EFSA GMO Panel considers that the 2006/2007, 2007 and 2007/2008 field trials are not appropriate to study unintended differences in the agronomic, phenotypic and compositional characteristics of soybean BPS-CV127-9 potentially caused by the transformation process. In spite of these limitations, data from the above mentioned trials remain informative for the general assessment of the GM soybean characteristics in terms of growth habit, vegetative vigour, phenology, reproductive behaviour and stress susceptibility.

- The two additional field trials were performed according to the requirements outlined in EFSA (2011a) and formed the basis for the current comparative assessment.

- In the field trial performed in Brazil in 2009/2010 (seven locations),[22] soybean BPS-CV127-9 was grown together with its conventional counterpart Conquista and three non-GM soybean reference varieties in each of the seven locations, with four replications per location. Each block included soybean BPS-CV127-9 treated either with a target herbicide (imazapyr) or with

[17] Dossier: Part I—Sections D7.1.5, D9.1, D9.2 and Annex 9; additional information: 03/11/2011 (Appendix 4), 09/08/2012 (Appendices 1 and 2).
[18] Dossier: Part I—Sections D7.1.5, D9.1, D9.2 and Annex 10; additional information: 03/11/2011 (Appendix 4), 09/08/2012 (Appendices 1 and 2).
[19] Dossier: Part I—Sections D7.1.5, D9.1, D9.2 and Annex 26.
[20] Additional information: 26/11/2010 (Appendix 4), 26/05/2011 (Appendix 3), 03/11/2011 (Appendix 4), 25/07/2012 (Appendix x), 09/08/2012 (Appendices 1 and 2), 04/02/2013 (Appendix 2), 13/05/2013 (Appendix 2).
[21] Additional information: 04/02/2013 (Appendix 2), 13/05/2013 (Appendix 2).
[22] Additional information: 26/11/2010 (Appendix 4), 26/05/2011 (Appendix 3), 03/11/2011 (Appendix 4), 25/07/2012 (Appendix x), 09/08/2012 (Appendices 1 and 2), 04/02/2013 (Appendix 2), 13/05/2013 (Appendix 2).

conventional herbicides, as well as the remaining test materials (conventional counterpart, the negative segregant and reference varieties) treated with the conventional herbicides.

- In the field trial performed in the USA in 2011,[23] soybean BPS-CV127-9 was grown at one site in Georgia together with its conventional counterpart Conquista and four non-GM soybean reference varieties in four replications.

The EFSA GMO Panel considers that Conquista had a genetic background comparable to that of the line of soybean BPS-CV127-9, as evidenced by the corresponding pedigree/breeding tree. Therefore, Conquista is regarded as an appropriate conventional counterpart.

Table 1: Overview of comparative assessment studies with soybean BPS-CV127-9 provided with application EFSA-GMO-NL-2009-64

Study focus	Study type	Study details	Comparators		Non-GM soybean reference varieties
			Conventional counterpart	Negative segregant	
General agronomic and phenotypic characteristics and composition	Field	2006–2007,Brazil (seven locations)[24]	None	1	2
		2007,Brazil (six locations)[25]	None	1	2
		2007–2008,Brazil (seven locations)[26]	None	1	2
		2009–2010,Brazil (seven locations)[27]	Conquista	1	6
		2011,USA (one location)[28]	Conquista	1	4
Pollen characteristics	Laboratory	Greenhouse-collected pollen[29]	None	1	None
Nodulation characteristics	Field	2006–2007, 2007 and 2007–2008,Brazil (20 locations)[30]	None	1	6
Seed characteristics	Laboratory	Field-collected seed[31]	None	1	6

4.1.2. Agronomic and phenotypic analysis

In the 2009/2010 and 2011 field trials, information on phenotypic and agronomic characteristics of soybean BPS-CV127-9 and its conventional counterpart was generated to compare their growth habit, vegetative vigour and reproduction characteristics. The same GM soybean variety used in Brazil was used for the single US site. The EFSA GMO Panel noticed that the values observed, for both soybean BPS-CV127-9 and the conventional counterpart, for the endpoints days to maturity, germination, yield, initial plant stand and plant height, were very different in the seven Brazilian sites from those of

[23] Additional information: 04/02/2013 (Appendix 2), 13/05/2013 (Appendix 2).
[24] Dossier: Part I—Sections D7.1.5, D9.1, D9.2 and Annex 9; additional information: 03/11/2011 (Appendix 4), 09/08/2012 (Appendices 1 and 2).
[25] Dossier: Part I—Sections D7.1.5, D9.1, D9.2 and Annex 10; additional information: 03/11/2011 (Appendix 4), 09/08/2012 (Appendices 1 and 2).
[26] Dossier: Part I—Sections D7.1.5, D9.1, D9.2 and Annex 26.
[27] Additional information: 26/11/2010 (Appendix 4), 26/05/2011 (Appendix 3), 03/11/2011 (Appendix 4), 25/07/2012, 09/08/2012 (Appendices 1 and 2), 04/02/2013 (Appendix 2), 13/05/2013 (Appendix 2).
[28] Additional information: 04/02/2013 (Appendix 2), 13/05/2013 (Appendix 2).
[29] Dossier: Part I—Sections D7.1.5, D9.1, D9.2 and Annex 14.
[30] Dossier: Part I—Sections D7.1.5, D9.1, D9.2 and Annexes 9, 10, 26.
[31] Dossier: Part I—Sections D7.1.5, D9.1, D9.2 and Annexes 9, 10, 26.

the single field trial site in the USA. Although this US site is located in a zone with the same maturation category (zone VIII), the EFSA GMO Panel considers that these differences may have arisen because the chosen soybean BPS-CV127-9 variety and its conventional counterpart were not well adapted to the US local agronomic and environmental conditions. Furthermore, the US field trial was conducted later in the season, resulting in a delayed maturation and harvesting time (two months) of soybean BPS-CV127-9 and its conventional counterpart compared with the non-GM soybean reference varieties.[32] Owing to this delay in harvesting of soybean BPS-CV127-9 and its conventional counterpart in the US site, the test of equivalence could be applied only to the endpoints measured before harvesting and not to those measured after harvesting, such as yield and seed weight.

The statistical analysis of the data collected from the eight field trial sites (seven in Brazil and one in the USA) showed significant differences for the following endpoints: germination, initial plant stand, plant height, yield and seed weight. For the endpoints measured before harvesting, the test of equivalence indicated that all the observed differences fell within the equivalence limits set by the non-GM soybean reference varieties (equivalence category I).[33] For the two endpoints measured after harvesting (yield and seed weight), the applicant claimed similar conclusions for the test of equivalence. However, owing to the difference in harvesting time of the non-GM soybean reference varieties compared with soybean BPS-CV127-9 and its conventional counterpart, the EFSA GMO Panel considers that the conclusion for the endpoints yield and seed weight cannot be supported by the data. Moreover, several of the endpoints listed above (germination, initial plant stand, plant height, yield and seed weight) showed statistically significant differences when the Brazilian sites were analysed independently from the US site. In particular, seed weight values of soybean BPS-CV127-9 were consistently higher than those of its conventional counterpart and non-GM soybean reference varieties, whereas yield values were always lower. Non-equivalence was observed for seed weight when treated with intended herbicide (equivalence category IV) and when treated with the conventional herbicides (equivalence category III). Therefore, the observed differences required further assessment.

As yield components are known to be correlated, the higher seed weight values observed for soybean BPS-CV127-9 could be explained by a lower number of seeds per square metre, which is expected in the case of lower germination rate and initial plant stand count. Therefore, although the EFSA GMO Panel considers that the occurrence of an unintended effect in seed weight cannot be excluded, the observed differences in several endpoints (including seed weight) may equally point to poor crop development.

In the 2006/2007, 2007 and 2007/2008 field trials, statistically significant differences were reported between soybean BPS-CV127-9 and a negative segregant for some endpoints at specific locations. All values were within the range of the non-GM soybean reference varieties, suggesting that soybean BPS-CV127-9 shows similar agronomic performances as non-GM soybean reference varieties.

Following a request of the EFSA GMO Panel, the applicant supplied additional information on mean seed weight (size) of other soybean varieties bearing event BPS-CV127-9.[34] Although these data seem to indicate that introgression of event BPS-CV127-9 into a variety does not affect seed size, the EFSA GMO Panel considers that the occurrence of an unintended effect in seed weight cannot be ruled out. The potential consequences of the observed difference in seed weight are further discussed in Section 6.

The applicant also reported data on nodulation, pollen and seed germination/quality characteristics of soybean BPS-CV127-9. However, the EFSA GMO Panel considers these data to be inconclusive for the comparative assessment for the following reasons:

[32] Additional information: 26/03/2013 (spontaneous submission).
[33] Additional information: 04/02/2013 (Appendix 2), 13/05/2013 (Appendix 2).
[34] Additional information : 09/08/2012 (Appendices 1 and 2), 26/03/2013 (spontaneous submission).

- Nodulation characteristics were measured in field trials which did not include a proper comparator. Moreover, the pre-sowing application of nitrogen fertiliser along with a mix of herbicides, insecticides and fungicides reduced the contribution of the symbiotic nitrogen fixation.

- Pollen characteristics of soybean BPS-CV127-9 were not compared with those of an appropriate comparator. In addition, no non-GM soybean reference varieties were included in the assessment.

- Seed germination and quality characteristics of soybean BPS-CV127-9 were not compared with those of an appropriate comparator. In addition, no non-GM soybean reference varieties were included in the assessment.

4.1.3. Compositional analysis[35]

The compositional studies of soybean seeds were based on the field trials in Brazil in the season 2009/2010 and in the USA in 2011, as these field trials included the conventional counterpart Conquista.

The 82 parameters[36] measured were in agreement with the OECD consensus document on key compositional considerations for new varieties of soybean (OECD, 2001). In accordance with the type of genetic modification, additional parameters were included in the compositional analysis (i.e. free amino acids). Ten analytes (free cysteine, free tyrosine, palmitoleic acid 16:1, vitamin B2-riboflavin, vitamin B3-niacin, arachidic acid 20:0, behenic acid 22:0, eicosenoic acid 20:1, margaric acid 17:0 and myristic acid 14:0) had 40 % or more sample values below the limit of quantification and were excluded from the statistical analysis.

Plants sprayed with maintenance pesticides showed statistically significant differences between soybean BPS-CV127-9 and its conventional counterpart for 42 of the 72 compounds. The test of equivalence indicated that the differences identified for 41 of these 42 compounds fell within the equivalence limits set by the non-GM reference varieties (equivalence categories I or II) included in the study and therefore did not need further assessment. Equivalence could not be established for δ-tocopherol (equivalence category III). The level of δ-tocopherol in soybean BPS-CV127-9 was higher than in the conventional counterpart and also slightly higher than in the non-GM soybean reference varieties (non-sprayed GM: 8.1 ± 1.3 mg/100 g; conventional counterpart: 7.2 ± 1.5 mg/100 g). The EFSA GMO Panel noted that, in the literature, the range of δ-tocopherol levels across different soybean varieties can vary up to 3.3 fold (Carrão-Panizzi and Erhan, 2007). As tocopherols are desired constituents in soybean and their levels were within the range reported in conventional soybeans, the EFSA GMO Panel identified no need for further assessment.

Samples sprayed with the intended herbicide in addition to the maintenance herbicide showed statistically significant differences for 38 compounds in soybean seeds. The test of equivalence indicated that the differences identified for 37 of these 38 compounds fell within the equivalence limits set by the non-GM reference varieties (equivalence categories I or II) included in the study and

[35] Dossier: Part I—Section D7.1.

[36] The parameters measured were ash, crude fat, moisture, protein, total carbohydrates, acid detergent fibre, crude fibre, neutral detergent fibre, total dietary fibre, alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, free alanine, free arginine, free aspartic acid, free cysteine, free glutamic acid, free glycine, free histidine, free isoleucine, free leucine, free lysine, free methionine, free phenylalanine, free proline, free serine, free threonine, free tyrosine, free valine, arachidic acid 20:0, behenic acid 22:0, eicosenoic acid 20:1, linoleic acid 18:2, linolenic acid 18:3, margaric acid 17:0, myristic acid 14:0, oleic acid 18:1, palmitic acid 16:0, palmitoleic acid 16:1, stearic acid, total diadzein, total genistein, total glycitein, calcium, iron, magnesium, phosphorus, potassium, phosphatidic acid, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, lectin, phytic acid, raffinose, stachyose, trypsin inhibitor, urease, α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol, total tocopherols, vitamin B1-thiamine, vitamin B2-riboflavic acid, vitamin B3-niacin and vitamin B9-folic acid.

therefore did not need further assessment. As in the soybean material not sprayed with the target herbicide, δ-tocopherol fell within equivalence category III. The increased level of δ-tocopherol (sprayed GM: 8.3 ± 1.2 mg/100 g) is discussed above.

Compositional data on soybean forage were only available from the field trials performed in 2006/2007 and 2007, where negative segregants were used as the only comparators. Although the GMO Panel could not conclude on the absence of unintended effects, no direct effect was observed.

4.2. Conclusion

Based on the available information on the agronomic and phenotypic characteristics of soybean BPS-CV127-9, the EFSA GMO Panel concludes that there is a difference in seed weight which needs further assessment (Section 6). The EFSA GMO Panel also concludes that no differences requiring further assessment were identified in the composition of seeds obtained from soybean BPS-CV127-9. 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.

5. Food/feed safety assessment

5.1. Evaluation of relevant scientific data

5.1.1. Effect of processing[37]

Soybean BPS-CV127-9 will be used for the production and manufacturing of food and feed products in the same way as any other commercial soybean variety. As no biologically relevant compositional differences raising safety concerns were identified in seeds, the effect of processing on soybean BPS-CV127-9 is not expected to be different compared with that on conventional soybean.

The AtAHAS protein was not detectable in any of these processed soybean fractions by ELISA.[38]

5.1.2. Toxicology[39]

5.1.2.1. Protein used for safety assessment

Owing to the low expression levels of the AtAHAS protein in tissues of soybean BPS-CV127-9 (below the level of quantification in seeds, Section 3.1.3) and the difficulty of isolating a sufficient quantity of purified protein from soybean BPS-CV127-9, the EFSA GMO Panel accepts the use of a recombinant AtAHAS protein produced in *Escherichia coli* for safety testing.

The *E. coli*-produced AtAHAS protein, designed as lot AtAHAS-0107, was characterised biochemically and immunologically. The lot AtAHAS-0107 was shown by ELISA to contain approximately 52.4 % AtAHAS, corresponding to approximately 90.6 % of the total protein in the preparation. The molecular weight, about 64 kDa, was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and the amino acid sequences of the AtAHAS protein and its internal peptide fragments were determined by N-terminal sequencing. Western blot analysis using polyclonal antibodies specific for the AHAS protein confirmed its immunoreactivity. An enzymatic assay confirmed the active catalytic function of the *E. coli*-produced AtAHAS protein, and the reduced sensitivity to inhibition by imazethapy, an imidazolinone herbicide, further confirmed the identity of the *E. coli*-produced AtAHAS. The solubility of the *E. coli*-produced AtAHAS protein in different buffers was also determined.[40]

[37] Dossier: Part I—Section D7.1.6.
[38] Dossier: Part I—Annex 13.
[39] Dossier: Part I—Section D7.2.
[40] Dossier: Part I—Annex 15.

The chemical and functional equivalence between the *E. coli*-produced AtAHAS protein (lot AtAHAS-0107) and the plant protein purified from young leaf tissue of soybean BPS-CV127-9 plants was demonstrated by molecular weight determination, N-terminal sequencing of the full-length protein and its internal peptide fragments, immunoreactivity with AHAS-specific antibody, enzymatic activity and the inhibition of the activity by imidazolinone herbicide and feedback inhibition by branched-chain amino acids, glycosylation.[41]

Considering that the AtSEC61γ protein was below the level of detection (section 3.1.3), and the high homology of the AtSEC61γ protein to the endogenous SEC61γ protein (93 % identity at the amino acid sequence level),[42] the EFSA GMO Panel is of the opinion that further safety assessment is not needed.

5.1.2.2. Toxicological assessment of the newly expressed protein

The assessment of potential toxicity of the newly expressed proteins present within soybean BPS-CV127-9 includes:

(a) Bioinformatic studies

Bioinformatic analyses[43] of the amino acid sequences of the AtAHAS and AtSEC61γ proteins in soybean BPS-CV127-9 revealed no significant similarities to known toxic proteins.

(b) Resistance to degradation by proteolytic enzymes[44]

The resistance to degradation by pepsin of the AtAHAS proteins, extracted from the plant and *E. coli*-produced proteins, was tested in solutions at pH 1.2. The integrity of the test protein in samples of the incubation mixture taken at various time points was analysed by SDS-PAGE followed by protein staining and Western blotting. The *E. coli*-produced AtAHAS protein was degraded within 30 seconds, whereas the plant extracts were degraded within 2 minutes. Some low molecular weight bands of the *E. coli*-produced AtAHAS protein (less than 6 kDa) were observed by Coomassie blue.

The resistance to degradation by pancreatin of the AtAHAS proteins were also studied in solutions at pH 7.5. The integrity of the test protein in incubation mixture samples taken at various times was analysed by SDS-PAGE followed by protein staining and Western blotting. Stable fragments of different molecular weights in leaf and grain extracts were observed by Western blotting. The applicant provided additional information[45] showing that the same stable fragments were visible in both the protein extracts of soybean BPS-CV127-9 and the protein extracts of its conventional counterpart. The EFSA GMO Panel notes that the resistance to degradation by pancreatin is not required by either the EFSA guidance document (EFSA, 2006a) or Codex Alimentarius (CAC, 2009).

The applicant also studied the resistance to degradation by pepsin of the AtSEC61γ protein in solutions at pH 1.2. The integrity of the test protein in the incubation mixture samples taken at various times was analysed by SDS-PAGE followed by Western blotting. No intact protein was visible after 30 seconds of incubation.

(c) Acute oral toxicity testing

The potential acute oral toxicity of AtAHAS was tested in mice (strain CD-1). There were no adverse effects after administration of a single oral dose of AtAHAS (lot AHASL-0107) at 5 000 mg/kg body weight (bw)/day (corresponding to 2 620 mg full-length AtAHAS/kg bw).[46] The EFSA GMO Panel is

[41] Dossier: Part I—Annex 16.
[42] Dossier: Part I—Annex 6.
[43] Dossier: Part I—Annex 20; additional information: 04/02/2013.
[44] Dossier: Part I—Section D.7.3; additional information: 16/04/2013.
[45] Additional information: 16/04/2013.
[46] Dossier: Part I—Annex 21.

of the opinion that acute toxicity testing of the newly expressed proteins is of little additional value for the risk assessment of the repeated consumption of food and feed from GM plants by humans and animals.

(d) Repeated-dose toxicity study

Considering that (1) the expression of the AtAHAS protein was below the limit of quantification in all grain samples from both soybean BPS-CV127-9 and its conventional counterpart (Section 3.1.3); (2) the AHAS family of proteins is ubiquitous in living organisms and human and animals are continuously exposed to them; and (3) there is a lack of relevant similarities of AtAHAS with any toxins in bioinformatic studies; the EFSA GMO Panel does not see a need for a repeated-dose toxicity study.

5.1.2.3. Toxicological assessment of new constituents other than proteins and/or changed levels of natural constituent

No new constituents other than AtAHAS protein were deliberately introduced and expressed in soybean BPS-CV127-9. No relevant changes in the composition of soybean BPS-CV127-9 were detected in the comparative compositional analysis (Section 4.2.2). Therefore, a toxicological assessment of new constituents is not applicable.

5.1.3. Animal studies with the food/feed derived from soybean BPS-CV127-9

(a) Sub-chronic toxicity study

A 90-day study[47] was carried out with Wistar Crl:Wi(Han) rats using a protocol adapted from OECD guideline 408. Groups of 10 males and 10 females (two replicates per sex) received *ad libitum* diets containing 11 % or 33 % (w/w) toasted meal. The meal was derived from the seed of soybean BPS-CV127-9 and of its conventional counterpart (Conquista), and from two commercial non-GM soybean varieties (Monsoy 8001, Coodetec 217). Diets were uniformly balanced with respect to nutrients and caloric values.

Animals were housed in cages with five rats of the same sex per cage and, in the data analysis, the individual animal was considered the experimental unit, ignoring possible biases owing to cage interactions. As the cage should have been considered the experimental unit and as the number of experimental units per treatment was low (two per sex), the EFSA GMO Panel did not consider this study in its evaluation.

(b) Chicken study[48]

The applicant provided a 42-day broiler feeding study in which the growth of birds given diets containing soybean meal produced from soybean BPS-CV127-9 was compared with that of birds given the meal prepared from the conventional counterpart 'Conquista' and two commercial non-GM soybean varieties (Monsoy 8001, Coodetec 217). A total of 576 day-old broilers (Ross 508) were assigned to one of the four diet treatments, each treatment consisting of 12 replicates of 12 birds (six males and six females). Preliminary experiments established the apparent metabolisable energy (AMEn) and amino acid digestibility for each soybean meal. Diets were formulated to be iso-energetic and to have equal protein/amino acid digestibility. The soybean content varied according to the age of the birds, starting with 40 % for the first 10 days of feeding, reducing to 30 % during the final stages of growth. Body weight, weight gain, feed intake and feed-to-gain ratio was measured at days 10, 28, 35 and 42. Analysis of variance found no interaction between treatment and sex. A Dunnett test was used to compare the results from the animals fed soybean meal from BPS-CV127-9 soybean with

[47] Additional information: 13/11/2012, 13/05/2013, also published as (Chukwudebe et al., 2012).
[48] Dossier: Part I—Annex 23.

those from the animals fed the soybean meals from the conventional counterpart and the two commercial varieties.

No significant differences were found for any of the measured parameters between BPS-CV127-9, its conventional counterpart and one of the commercial varieties. However, birds given the soybean meal prepared from Coodetec 217 showed significantly poorer performance throughout the trial when compared with all other birds, as a result of reduced feed intake. All Coodetec 217 soybean samples were tested for the most commonly encountered mycotoxins, the most likely explanation for reduced intake. However, none was detected and the reason for reduced intake compared with the other diets remains unexplained.

The growth of birds given diets containing soybean meal produced from soybean BPS-CV127-9 did not differ significantly from birds given meal prepared from other soybean varieties, including the conventional counterpart Conquista, as expected from the compositional data. The trial demonstrates that no unintended effects of a magnitude sufficient to affect performance have been introduced by the genetic modification.

5.1.4. Allergenicity[49]

The strategies used when assessing the allergenic potential focus on the characterisation of the source of the recombinant protein, on the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons and on whether the transformation has altered the allergenic properties of the modified plant.

5.1.4.1. Assessment of allergenicity of the newly expressed protein

A weight-of-evidence approach is followed, taking into account all the information obtained with various test methods, as no single experimental method yields decisive evidence for allergenicity (EFSA 2006a, 2011a; CAC, 2009).

The *csr1-2* gene and the *AtSEC61γ* gene present in soybean BPS-CV127-9 originate from *A. thaliana*, which is not considered to be a common allergenic source. The newly expressed protein AtAHAS is present in grain with a level of expression below the limit of quantification. The AtSEC61γ protein was below the level of detection (Section 3.1.3).

Updated bioinformatic analyses[50] of the amino acid sequence of the AtAHAS and AtSEC61γ proteins, using the criterion of 35 % identity in a window of 80 amino acids, revealed no significant similarities to known allergens. In addition, analyses searching matches of eight contiguous identical amino acid sequences between the AtAHAS and AtSEC61γ proteins and known allergens performed by the applicant confirmed the outcome of the previous bioinformatic analyses.

The studies on resistance to proteolytic enzymes of the newly expressed proteins are described in section 5.1.2.2.

Based on all the information available, the EFSA GMO Panel considers that there are no indications that the AtAHAS protein in soybean BPS-CV127-9 may be allergenic in the intended conditions of use.

5.1.4.2. Assessment of allergenicity of the whole genetically modified plant

According to the EFSA GMO Panel guidance document (EFSA, 2006a, 2011a), when the plant receiving the introduced gene is known to be allergenic, the applicant should test any potential change

[49] Dossier: Part I—Section D7.9.
[50] Dossier: Part I—Section D7.3.1; additional information: 04/02/2013.

in the allergenicity of the whole GM plant by comparing the allergen repertoire with that of its appropriate comparator(s). In this context, soybean is considered a common allergenic food.[51]

Initially, the applicant provided 2D-PAGE gels followed by silver staining for the protein detection of extracts of soybean BPS-CV127-9 and its conventional counterpart. Using image analysis, the applicant quantified some of the spots (corresponding to known allergens) for both of the extract samples. The applicant provided additional information[52] where the identities of the quantified allergen spots were confirmed by mass spectrometry analysis.

The applicant also performed 1D-PAGE followed by staining and Western blotting (using sera from ten individuals allergic to soybean).[53] In addition, an immunoglobulin E (IgE) inhibition ELISA study using pooled sera from individuals allergic to soybean was performed. The EFSA GMO Panel has previously presented the limitations of the 1D-PAGE gels and the use of pooled sera for the allergenicity assessment (see Annex 4 and Annex 5 of EFSA, 2010). Therefore, the EFSA GMO Panel could not use the 1D-PAGE and Western blotting studies with pooled sera to complement the previous 2D-PAGE study.

On request of the EFSA GMO Panel, the applicant provided additional information[54] using individual sera from eight subjects with clinically confirmed allergy to soybeans and sera from negative controls. In the 2D immunoblot analysis, no meaningful differences in the IgE binding patterns between extracts of soybean BPS-CV127-9 and its conventional counterpart were detected.

In the context of the present application and based on all the available information, the EFSA GMO Panel concludes that there are no indications that the genetic modification might significantly change the overall allergenicity of soybean BPS-CV127-9 when compared with that of its conventional counterpart.

5.1.5. Nutritional assessment of genetically modified food/feed derived from genetically modified plants[55]

The intended trait of soybean BPS-CV127-9 is herbicide tolerance, with no intention to alter the nutritional parameters. The compositional data, indicating nutritional equivalence between soybean BPS-CV127-9, its conventional counterpart and non-GM reference soybean varieties (Section 4.1.2), was confirmed by a feeding study with broilers (Section 5.1.3.b).

In accordance with the EFSA guidance document (EFSA, 2006a, 2011), the EFSA GMO Panel concludes that the data provided indicate that diets formulated with defatted soybean meal derived from soybean BPS-CV127-9 are as nutritious as those formulated with defatted soybean meal derived from commercial non-GM soybean varieties.

5.1.6. Post-market monitoring of genetically modified food/feed

No data indicating that soybean BPS-CV127-9 is any less safe than its conventional counterpart have emerged. In addition, soybean BPS-CV127-9 is as nutritious as non-GM soybean varieties. Therefore, in line with the guidance documents (EFSA, 2006a, 2011a), the EFSA GMO Panel is of the opinion that post-market monitoring of the GM food/feed is not necessary.

[51] Directive 2007/68/EC of the European Parliament and of the Council of 27 November 2007 amending Annex IIIa to Directive 2000/13/EC of the European Parliament and of the Council as regards certain food ingredients. OJ L 310, 27.11.2007, p. 11–14.
[52] Additional information: 25/07/2012.
[53] Additional information: 26/112010.
[54] Additional information: 26/05/2011.
[55] Dossier: Part I—Section D7.2.5.

5.2. Conclusion

No indications that the AtAHAS protein expressed in soybean BPS-CV127-9 may be allergenic or toxic and that the overall allergenicity of the whole plant has been changed were found. No biologically relevant differences were identified in the nutritional characteristics of soybean BPS-CV127-9 compared with its conventional counterpart. The EFSA GMO Panel concludes that soybean BPS-CV127-9 is as safe and nutritious as non-GM soybean varieties.

6. Environmental risk assessment and monitoring plan

6.1. Evaluation of relevant scientific data

The scope of application EFSA-GMO-NL-2009-64 is for import, processing and food and feed uses and does not include cultivation of soybean BPS-CV127-9. Therefore, the environmental risk assessment is concerned with the accidental release into the environment of soybean BPS-CV127-9 seeds (i.e. during transport and/or processing) and with indirect exposure, mainly through ingestion by animals and their manure and faeces leading to exposure of the gastrointestinal tract and soil microorganisms.

6.1.1. Environmental risk assessment

6.1.1.1. Potential unintended effects on plant fitness due to the genetic modification[56]

The applicant presented agronomic and phenotypic data on soybean BPS-CV127-9 gathered from field trials conducted in soybean growing areas in Brazil during the growing seasons 2006/2007, 2007, 2007/2008 and 2009/2010, and in the USA during the growing season 2011 (Section 4.1.2). Seed characteristics of soybean BPS-CV127-9 were also evaluated under growth chamber conditions. Although uncertainty on the exact cause of the observed difference in seed weight remains (Section 4.1.1), the dataset does not show major phenotypic changes from the conventional counterparts and/or reference varieties indicating altered fitness, persistence and invasiveness of soybean BPS-CV127-9 plants, unless these plants are exposed to imidazolinone-containing herbicides.

Moreover, the EFSA GMO Panel is not aware of any scientific report of increased spread and establishment of soybean BPS-CV127-9 or soybean with comparable properties, or of any change in survival capacity, including overwintering (Dorokhov et al., 2004; Owen, 2005; Kim et al., 2006; Bagavathiannan and Van Acker, 2008; Lee et al., 2009).

It is considered very unlikely that the spread, establishment and survival of soybean BPS-CV127-9 would be increased as a result of the herbicide tolerance trait. This trait can only be regarded as providing a potential agronomic and selective advantage to soybean BPS-CV127-9 when exposed to imidazolinone-containing herbicides. In addition, there is no evidence suggesting that larger soybean seeds would have a greater persistence and invasiveness potential than smaller ones. This is because the establishment and survival of soybean outside cultivation areas is limited by a combination of low competitiveness, absence of a dormancy phase and susceptibility to plant pathogens and frost, and that it is unlikely that these factors are affected by the size of soybean seeds.[57] Moreover, cultivated soybean seeds rarely display any dormancy characteristics and, only under certain environmental conditions, can grow as volunteers in the year following cultivation (OECD, 2000). In fields, soybean seeds usually do not survive during the winter owing to predation, rotting, germination resulting in death or management practices prior to planting the subsequent crop. In the case of soybean volunteers occurring, these would not be particularly competitive (Owen, 2005).

The EFSA GMO Panel considers that, in the context of the scope of this application, the differences observed in seed weight are unlikely to significantly affect the overall fitness, invasiveness or

[56] Dossier: Part I—Sections D7.1.5, D9.1, D9.2; additional information: 03/11/2011.
[57] Additional information: 25/07/2012.

weediness of the soybean BPS-CV127-9. Therefore, the accidental release of soybean BPS-CV127-9 seeds (i.e. during transport and/or processing) would not result in the establishment of plants exhibiting dissemination capabilities different from existing conventional soybean varieties and would not create additional agronomic or environmental impacts.

The EFSA GMO Panel concludes that, considering the scope of this application, the available data and the poor ability of soybean to survive outside cultivated land, there is very little likelihood of any environmental effects owing to the accidental release into the environment of viable seeds from soybean BPS-CV127-9.

6.1.1.2. Potential for gene transfer[58]

The EFSA GMO Panel evaluated the potential for horizontal and vertical gene flow of soybean BPS-CV127-9, as well as the potential environmental consequences of such gene transfer. 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 the dispersal of pollen and seeds.

(a) Plant-to-bacteria gene transfer

Genomic plant DNA is a component of several food and feed products derived from soybean. It is well documented that DNA present in food and feed becomes substantially degraded during processing and digestion in the human or animal gastrointestinal tract. However, a low level of exposure of fragments of ingested DNA, including the recombinant fraction of such DNA, to bacteria in the digestive tract of humans, domesticated animals and other environments exposed to the GM plant or plant material is expected.

Current scientific knowledge of recombination processes in bacteria suggests that horizontal transfer of non-mobile, chromosomally located DNA fragments between unrelated organisms (such as plants to bacteria) is not likely to occur at frequencies detectable under natural conditions (for further details, see EFSA, 2009).

A successful horizontal transfer would require stable insertion of the transgene sequences into a bacterial genome and a selective advantage to be conferred on the transformed host. The only mechanism known to facilitate horizontal transfer of non-mobile, chromosomal DNA fragments to bacterial genomes is homologous recombination. The similarity between the plant and bacterial sequences can be situated in the transgene itself or in the flanking regions. In the case of sequence similarity between the transgenic DNA and the natural variants of the gene in bacteria, recombination could result in a gene replacement in bacteria.

No bacterial sequences are present in the insert. The *ahasl* (referred to as *csr1-2*) gene in soybean BPS-CV127-9 derives from *A. thaliana*, and is under the control of a eukaryotic promoter with limited, if any, activity in prokaryotic organisms (Section 3.1.2; EFSA, 2009). Acetohydroxyacid synthetases (synonym: acetolactate synthetases) are widespread among bacteria. However, at the gene level, they are only remotely related to the *A. thaliana ahasl* gene, as only short stretches within the bacterial sequences are homologous to the inserted *ahasl* gene. Therefore, the likelihood of recombination can be expected to be negligible. Similarly, the likelihood of non-homologous (illegitimate) recombination, requiring similarity between the recombining DNA molecules, is extremely low. Illegitimate recombination events have not been detected in studies that have exposed bacteria to high concentrations of GM plant DNA (see EFSA, 2009).

There is no indication that the expression of the *ahasl* gene of soybean BPS-CV127-9, which is regulated by a eukaryotic plant promoter derived from *A. thaliana*, would provide a selective

[58] Dossier: Part I—Sections D2, D9.3; additional information: 03/11/2011.

advantage and confer a new trait to hypothetical bacterial recipients, considering the presence of genes encoding for acetolactate synthetases in environmental bacteria.

The unlikelihood of double homologous recombination, the wide environmental presence of genetically diverse natural bacterial acetohydroxyacid synthetases and the absence of a plausible selective advantage for the potential bacterial recipients suggest that it is highly unlikely that the recombinant DNA will transfer and establish in the genome of bacteria in the human and animal digestive tract or in the environment (EFSA, 2009).

The EFSA GMO Panel did not identify properties of the DNA inserted in soybean BPS-CV127-9 that would change its likelihood of horizontal transfer compared with other plant genes. A selective advantage of hypothesised rare horizontal transfer events into bacterial communities has not been identified. Therefore, the EFSA GMO Panel concludes that the recombinant DNA in soybean BPS-CV127-9 does not represent an environmental risk in relation to its potential for horizontal transfer to bacteria.

(b) Plant-to-plant gene transfer

Soybean is an annual, almost completely self-pollinating, crop which has a percentage of cross-pollination usually lower than 1 % (Weber and Hanson, 1961; Caviness, 1966; Ahrent and Caviness, 1994; 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 (Caviness, 1966; OECD, 2000). Pollination and fertilisation are usually accomplished before the flower opens. 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. Cross-pollination rates vary significantly depending upon the soybean variety, flower synchrony, environmental conditions, experimental design and presence of pollinators (Abrams et al., 1978; Gumisiriza and Rubaihayo, 1978; Kikuchi et al., 1993; Ahrent and Caviness, 1994; Ray et al., 2003; Lu, 2005). Pollinators such as honeybees are thought to mediate pollination, although soybeans are not as attractive to insects as many other plants (Jaycox, 1970; Erickson, 1975a, b, 1984; Abrams et al., 1978; Erickson et al., 1978; Ortiz-Perez et al., 2006, 2008). Based on field trial data and a wind tunnel experiment, Yoshimura (2011) concluded that wind-mediated pollination appears to be negligible, as little airborne pollen was observed in and around a soybean field. Most cross-pollination events occur within a few metres of the pollen source, and decrease rapidly as the distance from the pollen source increases (Caviness, 1966; Yoshimura et al., 2006; Abud et al., 2007).

The plant-to-plant gene transfer from soybean is restricted to cultivated soybean because wild soybean relatives are not present in the EU. The genus *Glycine* is divided into two distinct sub-genera: *Glycine* and *Soja*. Soybean belongs to the sub-genus *Soja*. The sub-genus *Glycine* contains 16 perennial wild species, whereas the cultivated soybean, *Glycine max*, and its wild and semi-wild annual relatives, *Glycine soja* and *Glycine gracilis*, are classified as members of the sub-genus *Soja* (OECD, 2000). Owing to the low level of genomic similarity among species of the genus *Glycine*, *Glycine max* can only cross with other members of the *Glycine* sub-genus *Soja*; species of the sub-genus *Soja* are capable of hybridising and the hybrid seed can germinate normally and produce plants with fertile pollen and seeds (Hymowitz et al., 1998; Abe et al., 1999; Nakayama and Yamaguchi, 2002; Lu, 2005; Mizuguti et al., 2009, 2010). *Glycine soja* and *Glycine gracilis* are indigenous to China, Taiwan, Korea, Japan, the Far East region of Russia, Australia, the Philippines and the South Pacific and they have not been reported in other parts of the world where cultivated soybean is grown (Dorokhov et al., 2004; Lu, 2005).

Considering the scope of this application and the physical characteristics of soybean seeds, a possible pathway of gene dispersal is from seed spills (i.e. during transport and/or processing) and, ultimately, pollen of occasional feral GM soybean plants originating from seed spills. Seed dispersal from occasional feral soybean BPS-CV127-9 by animals is not expected, as neither the pods nor the seeds have morphological characteristics that would facilitate animal transport (OECD, 2000).

The likelihood of cross-pollination between feral soybean BPS-CV127-9 plants resulting from seed spills and cultivated soybean is extremely low. Apart from seed production areas, feral soybean BPS-CV127-9 plants will not persist over time. Survival of soybean plants outside cultivation areas is mainly limited by a combination of low competitiveness, absence of a dormancy phase and susceptibility to plant pathogens and frost. As these general characteristics are unchanged in soybean BPS-CV127-9, herbicide tolerance is not likely to provide selective advantages outside cultivation in Europe. Therefore, as for any other soybean varieties, GM soybean plants are not likely to establish feral populations under European environmental conditions. Furthermore, cultivated soybean seeds rarely display any dormancy characteristics and only under certain environmental conditions grow as volunteers in the year following cultivation (OECD, 2000). In soybean fields, seeds usually do not survive during the winter owing to predation, rotting, germination resulting in death or management practices prior to planting the subsequent crop (Owen, 2005). Thus, the accidental release of soybean BPS-CV127-9 seeds (i.e. through seed spills) would not result in the establishment of plants exhibiting dissemination capabilities different from existing conventional soybean varieties and would not create additional agronomic or environmental impacts.

Even if transgene flow occurred between occasional feral soybean BPS-CV127-9 and cultivated soybean plants, a selective advantage would only occur when exposed to imidazolinone-containing herbicides.

In conclusion, as soybean BPS-CV127-9 has no altered survival, multiplication or dissemination characteristics, except when exposed to imidazolinone-containing herbicides, the EFSA GMO Panel considers that the likelihood of unintended environmental effects as a consequence of the spread of genes from this GM soybean in Europe will not differ from that of conventional soybean varieties.

6.1.1.3. Interactions of the genetically modified plant with target organisms[59]

Interactions of soybean BPS-CV127-9 with target organisms are not considered to be a relevant issue by the EFSA GMO Panel, as there are no target organisms.

6.1.1.4. Interactions of the genetically modified plant with non-target organisms[60]

Owing to the intended uses of soybean BPS-CV127-9, which exclude cultivation, and the low level of exposure to the environment, potential interactions of the GM plant with non-target organisms are not considered to be an issue by the EFSA GMO Panel.

6.1.1.5. Interactions with the abiotic environment and biochemical cycles[61]

Given the scope of this application, which excludes cultivation of soybean BPS-CV127-9, and the low level of exposure to the environment, potential interactions with the abiotic environment and biogeochemical cycles are not considered to be a relevant issue by the EFSA GMO Panel.

6.1.2. Post-market environmental monitoring[62]

The objectives of a post-market environmental monitoring 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 environmental risk assessment are correct; and (2) to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment which were not anticipated in the environmental risk assessment.

Monitoring is related to risk management, and thus a final adoption of the post-market environmental monitoring plan falls outside the mandate of EFSA. However, the EFSA GMO Panel gives its opinion

[59] Dossier: Part I—Section D9.4; additional information: 03/11/2011.
[60] Dossier: Part I—Section D9.5; additional information: 03/11/2011.
[61] Dossier: Part I—Sections D9.8, D10; additional information: 03/11/2011.
[62] Dossier: Part I—Section D11 and Annex 25.

on the scientific content of the post-market environmental monitoring plan provided by the applicant (EFSA, 2011b).

The potential exposure to the environment of soybean BPS-CV127-9 would be through ingestion by animals and their manure and faeces leading to exposure of the gastrointestinal tract and soil microbial populations to recombinant DNA, and through accidental release into the environment of GM soybean seeds during transport and/or processing. As the environmental risk assessment does not cover cultivation of soybean BPS-CV127-9 and identified no potential adverse environmental effects, no case-specific monitoring is necessary.

The post-market environmental monitoring plan proposed by the applicant includes (1) the description of an approach involving operators (federations involved in soybean 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 newly established by EuropaBio for the collection of the information recorded by the various operators; and (3) the use of networks of existing surveillance systems (Lecoq et al., 2007; Windels et al., 2008). The applicant proposes to submit a post-market environmental monitoring report on an annual basis and a final report at the end of the consent period.

The EFSA GMO Panel considers the scope of the post-market environmental monitoring plan provided by the applicant consistent with the scope of this application, as the environmental risk assessment does not cover cultivation of soybean BPS-CV127-9 and identified no potential adverse environmental effects. In addition, the EFSA GMO Panel acknowledges the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure in the case of accidental release of viable seeds of soybean BPS-CV127-9. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the post-market environmental monitoring plan.

6.2. Conclusion

In the case of accidental release into the environment of viable soybean BPS-CV127-9 seeds, there are no indications of an increased likelihood of spread and establishment of feral soybean BPS-CV127-9 plants, unless these plants are exposed to imidazolinone-containing herbicides. Given the scope of this application, only a low-level exposure of environmental bacteria, including those in the gastrointestinal tract, to recombinant DNA from soybean BPS-CV127-9 is expected. Risks associated with an unlikely, but theoretically possible, horizontal gene transfer from soybean BPS-CV127-9 to bacteria have not been identified. Considering the scope of this application, the risk to non-target organisms is extremely low owing to the rare occurrence of feral soybean plants and the low levels of exposure through other routes. Interactions with the biotic and abiotic environment are therefore not considered to be a relevant issue.

The scope of the post-market environmental monitoring plan provided by the applicant and the reporting intervals are in line with the scope of this application and the EFSA GMO Panel scientific opinions providing guidance on the post-market environmental monitoring of GM plants (EFSA, 2006b, 2011). In addition, the EFSA GMO Panel acknowledges the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure in cases of accidental release of viable seeds of soybean BPS-CV127-9.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The EFSA GMO Panel was requested to carry out a scientific assessment of soybean BPS-CV127-9 for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003.

The EFSA GMO Panel is of the opinion that the molecular characterisation data provided for soybean BPS-CV127-9 are sufficient to conclude on this part of the risk assessment evaluation. Bioinformatic analyses and genetic stability studies did not raise any safety issues. The levels of the AtAHAS protein in soybean BPS-CV127-9 have been sufficiently analysed.

No differences were identified in the compositional data of seeds obtained from soybean BPS-CV127-9 that would require further assessment with regard to safety by the GMO Panel. On the agronomic and phenotypic characteristics of soybean BPS-CV127-9, a difference in seed weight was identified. However, this difference does not affect the overall safety of soybean BPS-CV127-9. 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. There are no indications that the AtAHAS protein expressed in soybean BPS-CV127-9 may be allergenic or toxic, and that the overall allergenicity of the whole plant has been changed. No biologically relevant differences were identified in the nutritional characteristics of soybean BPS-CV127-9 compared with its conventional counterpart, as indicated by compositional data and the chicken feeding study. The EFSA GMO Panel concludes that soybean BPS-CV127-9 is as safe and nutritious as non-GM soybean varieties.

Considering the scope of this application, the environmental risk assessment is concerned with indirect exposure, mainly through ingestion by animals and their manure and faeces leading to exposure of the gastrointestinal tract and soil microorganisms, and with the accidental release into the environment of viable soybean BPS-CV127-9 seeds (i.e. during transport and/or processing). In the case of accidental release into the environment of viable seeds of soybean BPS-CV127-9, there are no indications of an increased likelihood of spread and establishment of feral soybean BPS-CV127-9 plants, unless these plants are exposed to imidazolinone-containing herbicides. Considering the scope of this application, interactions of soybean BPS-CV127-9 with the biotic and abiotic environment are not considered to be a relevant issue. Risks associated with an unlikely but theoretically possible horizontal gene transfer from soybean BPS-CV127-9 to bacteria have not been identified. The scope of the post-market environmental monitoring plan provided by the applicant and the reporting intervals are in line with the scope of this application and the EFSA GMO Panel scientific opinions providing guidance on the post-market environmental monitoring of GM plants. In addition, the EFSA GMO Panel acknowledges the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure in cases of accidental release of viable seeds of soybean BPS-CV127-9.

In conclusion, the EFSA GMO Panel considers that the information available for soybean BPS-CV127-9 addresses the scientific issues indicated by the guidance documents of the EFSA GMO Panel and the scientific comments raised by the Member States, and that soybean BPS-CV127-9, as described in this application, is as safe as its conventional counterpart and commercial soybean varieties with respect to potential effects on human and animal health and the environment in the context of its intended uses.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of the Netherlands, received on 15 January 2009, concerning a request for placing on the market of soybean BPS-CV127-9 in accordance with Regulation (EC) No 1829/2003.

2. Acknowledgement letter, dated 5 February 2009, from EFSA to the Competent Authority of the Netherlands.

3. Letter from EFSA to the applicant, dated 24 February 2009, requesting additional information under completeness check.

4. Letter from the applicant, received on 26 May 2009, providing additional information under completeness check.

5. Letter from EFSA to the applicant, dated 22 June 2009, requesting additional information under completeness check.

6. Letter from the applicant, received on 26 June 2009, providing additional information under completeness check.

7. Letter from EFSA to the applicant, dated 13 July 2009, delivering the 'Statement of Validity' for application EFSA-GMO-NL-2009-64, soybean BPS-CV127-9, submitted by BASF Plant Science under Regulation (EC) No 1829/2003.

8. Letter from the applicant, received on 17 July 2009, providing EFSA with an updated version of the application EFSA-GMO-NL-2009-64.

9. Letter from EFSA to the applicant, dated 21 September 2009, requesting additional information and stopping the clock.

10. Letter from the applicant, received on 26 November 2010, providing additional information.

11. Letter from EFSA to the applicant, dated 18 February 2011, requesting additional information.

12. Letter from the applicant, received on 26 May 2011, providing additional information.

13. Letter from EFSA to the applicant, dated 6 July 2011, restarting the clock.

14. Letter from EFSA to the applicant, dated 2 August 2011, requesting additional information and stopping the clock.

15. Letter from EFSA to the applicant, dated 24 August 2011, with request for additional information.

16. Letter from the applicant, received on 3 November 2011, providing additional information.

17. Letter from EFSA to the applicant, dated 17 February 2012, requesting additional information.

18. Letter from the applicant, received on 25 July 2012, providing additional information.

19. Letter from EFSA to the applicant, dated 13 June 2012, requesting additional information.

20. Letter from the applicant, received on 9 August 2012, providing additional information.

21. Letter from the applicant, spontaneously providing additional information on 13 November 2012.

22. Letter from the applicant, spontaneously providing additional information on 4 February 2013.

23. Letter from EFSA to the applicant, dated 19 February 2013, requesting additional information.

24. Letter from the applicant, received on 26 March 2013, providing additional information. At the same time, other additional information was spontaneously provided.

25. Letter from EFSA to the applicant, dated 27 March 2013, requesting additional information.

26. Letter from the applicant, received on 16 April 2013, providing additional information.

27. Letter from EFSA to the applicant, dated 11 April 2013, requesting additional information.

28. Letter from the applicant, received on 13 May 2013, providing additional information.

29. Letter from EFSA to the applicant, dated 18 November 2013, requesting additional information.

30. Letter from the applicant, received on 26 November 2013, providing additional information.

31. Letter from EFSA to the applicant, dated 29 November 2013, restarting the clock.

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