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SCIENTIFIC OPINION

Scientific Opinion on application EFSA-GMO-NL-2007-45 for the placing on the market of herbicide-tolerant, high-oleic acid, genetically modified soybean 305423 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Pioneer[1]

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

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ABSTRACT

Soybean 305423 was developed through particle bombardment and contains *gm-fad2-1* and *gm-hra* expression cassettes, conferring a high oleic acid profile and tolerance to acetolactate synthase (ALS)-inhibiting herbicides. Bioinformatic analyses and genetic stability studies did not raise safety issues. Levels of the GM-HRA protein in soybean 305423 have been sufficiently analysed. Soybean 305423 differs from the conventional counterpart in the seed fatty acid profile and for the presence of the GM-HRA protein. It is agronomically equivalent to non-GM reference soybeans. The safety assessment of GM-HRA identified no concerns regarding potential toxicity and allergenicity. There are no indications that the overall allergenicity of soybean 305423 has changed. Nutritional assessment on soybean 305423 oil and derived food products did not identify concerns on human health and nutrition. There are no concerns regarding the use of feeding stuffs derived from soybean 305423. There are no indications of an increased likelihood of establishment and spread of feral GM soybean plants. Environmental risks associated with an unlikely, but theoretically possible, horizontal gene transfer from soybean 305423 to bacteria have not been identified. Potential biotic and abiotic interactions of soybean 305423 were not considered to be an issue owing to the low level of environmental exposure. The post-market environmental monitoring plan is in line with the scope of soybean 305423. The EFSA GMO Panel considers that the information available for soybean 305423 addresses the scientific comments raised by the Member States and states that the soybean 305423, as described in the application, is as safe as its conventional counterpart with respect to potential effects on human and animal health and the environment in the context of the scope. The GMO Panel recommends a post-market monitoring plan, focusing on the collection of consumption data for the European population, for the marketed foods and feed.

[1] On request from the Competent Authority of the Netherlands for an application EFSA-GMO-NL-2007-45 submitted by Pioneer, Question No EFSA-Q-2007-122, adopted on 4 December 2013.

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KEY WORDS

GMO, soybean (*Glycine max*), herbicide tolerant, high oleic acid, RNAi, HRA, Regulation (EC) No 1829/2003

SUMMARY

Following the submission of an application (EFSA-GMO-NL-2007-45) under Regulation (EC) No 1829/2003 from Pioneer, 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, high-oleic acid genetically modified (GM) soybean 305423 (Unique Identifier DP-3Ø5423-1) for food and feed uses, import and processing.

In delivering its scientific opinion, the EFSA GMO Panel considered the application EFSA-GMO-NL-2007-45, additional information supplied by the applicant, scientific comments submitted by the Member States and relevant scientific publications. The scope of application EFSA-GMO-NL-2007-45 is for food and feed uses, import and processing of soybean 305423 within the European Union (EU) as any non-GM soybean, but excludes cultivation in the EU.

The EFSA GMO Panel evaluated soybean 305423 with respect to the scope and the appropriate principles described in its Guidance documents for the risk assessment of GM plants and derived food and feed and on the post-market environmental monitoring of GM plants (EFSA, 2006a, 2011a).The scientific assessment included molecular characterisation of the inserted DNA, the expression of the target protein and the high-oleic acid phenotype. An evaluation of the comparative analysis of composition and agronomic and phenotypic traits 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 quality. An evaluation of environmental impacts and of the post-market environmental monitoring plan was undertaken.

Soybean 305423 was transformed using a biolistic DNA delivery system and expresses the *Glycine max-hra* (*gm-hra*) gene conferring tolerance to acetolactate synthase (ALS)-inhibiting herbicides. Soybean 305423 also expresses a fragment of the endogenous *fad2-1* gene resulting, through RNA interference, in the silencing of the endogenous *fad2-1* gene, which leads to a decreased level of the omega-6 fatty acid desaturase and a high-oleic acid phenotype.

The molecular characterisation data establish that the genetically modified (GM) soybean 305423 contains four complete and/or partial copies of the *Glycine max-fad2-1* (*gm-fad2-1*) and *gm-hra* expression cassettes. No other parts of the plasmid used for transformation are present in the transformed plant except for a small, non-functional vector fragment which does not include the origin of replication or the hygromycin-resistance gene. Results of updated bioinformatic analyses of the 5′ and 3′ flanking sequences and open reading frames (ORFs) present in the junction regions and within the insert did not indicate a safety issue. One plant among the 1 100 tested had undergone recombination and, as a result, had lost the entire *gm-hra* cassette and portions of the promoter elements flanking the cassette. However, the EFSA GMO Panel is of the opinion that this recombination, leading to loss of the trait, raises no safety issue. The stability of the inserted DNA was sufficiently confirmed over several generations. The four insertions are genetically linked and behave as a single Mendelian locus.

The EFSA GMO Panel compared the compositional, agronomic and phenotypic characteristics of soybean 305423, with its conventional counterpart and non-GM reference soybean varieties and assessed all statistically significant differences between soybean 305423 and its conventional counterpart, for which equivalence with the non-GM reference varieties could not be established. The EFSA GMO Panel concludes that the composition of soybean 305423 differs from that of the conventional counterpart and that of non-GM reference varieties in its fatty acid profile, the newly expressed protein *max* herbicide-resistant ALS (GM-HRA), consistently with the objective of the modification as well as with the expression of the ALS enzyme of soybean 305423; differences in the minerals zinc and calcium and the isoflavone glycitin were also noted, and for these no further assessment was deemed necessary owing to their well-known biochemical roles and to the magnitude of the reported levels. The EFSA GMO Panel also concludes that no differences were identified in the agronomic and phenotypic characteristics that would require further assessment with regard to safety.

Full replacement of vegetable oils with oil derived from soybean 305423 would not change substantially the average intake of saturated fatty acids (SFA) and n-3 polyunsaturated fatty acids (PUFA), but would increase average intake of monounsaturated fatty acids (MUFA) and odd chain fatty acids, and decrease n-6 PUFA intake. These changes in the average intake are small and without impact on health and nutrition. The contribution of fatty acids from soybean 305423 in other soybean products to overall human exposure would be small and is not expected to affect the conclusion on human health and nutrition.

The safety assessment identified no concerns regarding the potential toxicity and allergenicity of the newly introduced GM-HRA protein. There are no indications that the genetic modification might significantly change the overall allergenicity of soybean 305423 when compared with that of its conventional counterpart.

Based on the results of studies in chickens for fattening, laying hens, pigs and rats, it is concluded that feeding stuffs derived from soybean 305423 are safe and as nutritious as those derived from other non-GM soybean varieties for all animal species.

Considering the intended altered soybean 305423 nutritional composition, a proposal for a post-market monitoring (PMM) plan needs to be provided by the applicant (EFSA, 2006b, 2011c). EFSA recommends that the PMM should focus on the collection of consumption data for the European population.

The scope of application EFSA-GMO-NL-2007-45 is for food and feed uses, import and processing and does not include cultivation. Therefore, there is no requirement for scientific information on possible environmental effects associated with the cultivation of soybean 305423 in Europe. There are no indications of an increased likelihood of establishment and spread of feral GM soybean plants in the case of accidental release into the environment of viable soybean 305423 grains during transport and processing for food and feed uses, except in the presence of ALS-inhibiting herbicides. Considering the scope of this application, potential biotic and abiotic interactions of soybean 305423 were not considered to be an issue by the EFSA GMO Panel. The unlikely but theoretically possible transfer of the recombinant gene from soybean 305423 to environmental bacteria does not raise safety concerns because no selective advantage will be conferred to the recipients. The scope of the post-market environmental monitoring plan provided by the applicant is in line with the scope of the application. Furthermore, the EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the post-market environmental monitoring plan.

In conclusion, the EFSA GMO Panel considers that the information available for soybean 305423 addresses the scientific issues indicated by the Guidance document of the EFSA GMO Panel and the scientific comments raised by the Member States, and that soybean 305423 is as safe as its conventional counterpart and is unlikely to have adverse effects on human and animal health and the environment in the context of the scope of this application.

Considering the altered composition and nutritional values of soybean 305423, the EFSA GMO Panel considered a specific labelling proposal provided by the applicant in accordance with Articles 13(2)(a) and 25(2)(c) of Regulation (EC) No 1829/2003. The applicant proposed that food and feed products within the scope of the application should be labelled as “genetically modified soybean with altered fatty acid profile”. The GMO Panel is of the opinion that the compositional data show that the fatty acid composition of seeds of soybean 305423 and derived oil has indeed been changed in relation to the conventional counterpart.

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BACKGROUND

On 18 June 2007, the European Food Safety Authority received from the Dutch Competent Authority an application (Reference EFSA-GMO-NL-2007-45) for authorisation of genetically modified (GM) soybean 305423 (Unique Identifier DP-3Ø5423-1) submitted by Pioneer within the framework of Regulation (EC) No 1829/2003 on GM food and feed.[4] After receiving the application EFSA-GMO-NL-2007-45 and in accordance with Articles 5(2)(b) and 17(2)b of 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. 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 22 October 2007, 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 Member States and the European Commission and consulted nominated risk assessment bodies of the Member States, including the national Competent Authorities within the meaning of Directive 2001/18/EC[5], 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 22 January 2008) within which to make their opinion known.

The EFSA GMO Panel carried out a scientific assessment of genetically modified (GM) soybean 305423 in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003, taking into account the appropriate principles described in the guidance documents of the Scientific Panel on Genetically Modified Organisms for the risk assessment of food and feed derived from genetically modified plants (EFSA, 2006a, 2011a). In addition, the scientific comments of Member States, the additional information provided by the applicant and relevant scientific publications were taken into consideration.

On 20 December 2007, 28 February 2008, 22 May 2008, 13 August 2008, 11 February 2009, 8 January 2010, 27 April 2010, 5 August 2010, 21 October 2010, 30 May 2012, 19 September 2012, 8 February 2013, 20 February 2013, 12 April 2013 and 26 June 2013 the EFSA GMO Panel requested additional information from the applicant. The applicant provided additional information on 12 February 2008, 28 April 2008, 4 July 2008, 24 November 2008, 19 March 2009, 6 October 2009, 9 February 2010, 9 June 2010, 21 September 2010, 9 February 2011, 14 July 2011, 28 November 2011, 17 January 2012, 5 March 2012, 5 June 2012, 31 October 2012, 2 May 2013, 28 June 2013 and on 3 October 2013. After receipt and assessment of the full data package, the GMO Panel finalised its risk assessment of soybean 305423.

In giving its opinion on soybean 305423 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 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).

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

5 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. Official Journal of the European Communities, L 106, 1–38.

TERMS OF REFERENCE

The EFSA GMO Panel was requested to carry out a scientific assessment of soybean 305423 (Unique Identifier DP-305423-1) 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 a scientific opinion on information required under Annex II to the Cartagena Protocol. The EFSA GMO Panel did consider if there is a need for a specific labelling in accordance with Articles 13(2)(a) and 25(2)(c) of Regulation (EC) No 1829/2003. However, it did not consider proposals for 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

Soybean 305423 has been developed to increase the oleic acid content of seeds with the objective of improving the oxidative stability of the oil as a result of the reduced polyunsaturated fatty acid (PUFA) content. Reduction in the expression of soybean enzyme omega-6 desaturase was achieved by introducing a fragment of the coding region of the corresponding gene (*gm-fad2-1*). An optimised soybean gene, *gm-hra*, that encodes acetolactate synthase (ALS) was also introduced into the soybean as a selectable marker conferring tolerance to ALS-inhibiting herbicides.

The *gm-fad2-1* gene fragment inserted in soybean 305423, under the control of a seed-preferred promoter, corresponds to the middle of the coding region of the soybean *fad2-1* gene. Transcription of this fragment results in silencing of the endogenous *fad2-1* gene and leads to a decreased level of the corresponding fatty acid desaturase. As a consequence, the conversion of oleic acid to linoleic acid is inhibited and the oleic acid level is elevated. Since linolenic acid is produced from linoleic acid, linolenic acid content is also decreased in soybean 305423.

The second modification in soybean 305423 confers tolerance to sulphonylureas and some other herbicide classes. ALS, also known as acetohydroxyacid synthase (AHAS), is the primary target for these herbicides.

Soybean 305423 will be used for the production of soybean products as any commercial soybean variety. The main product for human use is soybean oil. In addition, soybean is used for the production of soybean milk, soybean protein isolate, flour, sprouts, baked or roasted soybeans, tofu, soybean sauce and other products for human consumption. Dehulled, fat-extracted toasted soybean meal is used as a source of protein in animal feed, sometimes in combination with soybean hulls. There is also a limited direct use of full-fat soybeans as animal feed.

All food, feed and processed products derived from soybean 305423 are expected to replace a portion of similar products from commercial soybean. Oil from this soybean might also replace oils from sources other than soybean, particularly those used for commercial frying and spraying.

2. Issues raised by Member States

The issues raised by the Member States are addressed in Annex G of the EFSA overall opinion[6] and have been considered in this scientific opinion.

3. Molecular characterisation

3.1. Evaluation of relevant scientific data

3.1.1. Transformation process and vector constructs[7]

Soybean 305423 was developed by particle bombardment of secondary somatic embryos derived from explants of small, immature soybean seed of cultivar Jack. Two gel-purified linear DNA fragments, PHP19340A and PHP17752A, were co-bombarded into embryogenic soybean cultures. Both fragments were isolated from plasmids in which they were constructed. Complete sequences of the introduced fragments were provided.

Fragment PHP19340A (2 924 bp) contains a *gm-fad2-1* gene fragment (597 bp) from the coding region of the microsomal omega-6 desaturase gene 1 (*fad2-1*) of soybean; it does not code for a functional protein, but it is designed to silence the expression of the endogenous *fad2-1* gene. The

[6] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2007-122
[7] Technical dossier/Sections C and D1.

seed-preferred promoter (2 084 bp) and the terminator (196 bp) are from soybean Kunitz trypsin inhibitor gene 3 (*KTi*3).

Fragment PHP17752A (4 512 bp) contains the *gm-hra* gene (1 971 bp), a modified form of the endogenous soybean *als* gene, which encodes ALS (GM-HRA). The protein confers tolerance to ALS-inhibiting herbicides and was used as a selectable marker. Transcription is regulated by an S-adenosyl-L-methionine synthetase (SAMS) promoter and the *als* gene terminator, both from soybean.

3.1.2. Transgene constructs in the GM plant[8]

Molecular characterisation of soybean 305423 to evaluate the insert copy number, insert integrity and presence of plasmid backbone was conducted by Southern analysis and the results were confirmed by sequencing of the inserted DNA from soybean 305423. For Southern analysis, leaf samples from soybean 305423 T_4 (transgenic generation 4) and T_5 generations were analysed. The T_4 generation represents soybean 305423 obtained by four rounds of self-pollination of the original transformed Jack soybean line (T_0). The T_5 generation represents the following round of self pollination.

The number and identity of the inserts were investigated not only by Southern analysis, but also by cloning of the inserts from plasmid and cosmid libraries and by polymerase chain reaction (PCR) amplification. Southern analyses were complicated by the fact that all introduced material was of soybean origin. The analyses, including additional information provided by the applicant, showed the presence of four inserts and the absence of all elements from the plasmid backbone (e.g. the hygromycin resistance gene), except for a small, non-functional fragment.

Multiple intact and truncated copies of fragment PHP19340A are present in soybean 305423 that contain, in total, eight copies of the *KTi*3 promoter, seven copies of the *gm-fad2-1* gene fragment and five copies of the *KTi*3 terminator. A single intact copy of fragment PHP17752A is present in soybean 305423. Soybean 305423 has a rather complex insertion arrangement, containing the following four insertions:

Insertion 1: PHP19340A fragment with a truncated *KTi*3 terminator, intact *gm-fad2-1* gene fragment and intact *KTi*3 promoter; intact PHP19340A fragment; intact PHP17752A fragment; PHP19340A fragment with an intact *KTi*3 promoter and a truncated *gm-fad2-1* gene fragment; PHP19340A fragment with a truncated *KTi*3 promoter and truncated *gm-fad2-1* gene fragment.

Insertion 2: PHP19340A fragment with a truncated *KTi*3 promoter, intact *gm-fad2-1* gene fragment and intact *KTi*3 terminator.

Insertion 3: one copy of the *KTi*3 promoter with a non-functional 495 bp fragment of the plasmid backbone.

Insertion 4: two truncated PHP19340A fragments in an inverted repeat configuration, both with a truncated *KTi*3 promoter and intact *gm-fad2-1* gene fragment and *KTi*3 terminator.

From the 5′ flanking region of the inserts 1, 2, 3 and 4, 7 000 bp, 7 599 bp, 2 439 bp and 2 899 bp were sequenced, respectively. From the 3′ flanking region of the inserts 1, 2, 3 and 4, 2 524 bp, 2 737 bp, 2 287 bp and 2 149 bp were sequenced, respectively. In each case, BLASTn sequence analysis resulted in significant identities to public and proprietary soybean genomic sequences.

Open reading frames (ORFs) present in the junction regions and within the insert were analysed in soybean 305423.[9] All ORFs were considered from stop codon to stop codon. Similarities to known protein allergens and toxins were examined. No matches to known or putative allergens and no alignments to any known or putative toxins were found. The analysis also showed that there are no potential fusion proteins in soybean 305423 that raise safety concerns for human or animal health.

[8] Technical dossier/Section D2.
[9] Additional information, May 2013.

3.1.3. Information on the expression of the insert[10]

Northern analysis indicated an effective suppression of transcripts of both the endogenous *fad2-1* gene and the introduced *gm-fad2-1* gene fragment in soybean 305423 seeds. This was also reflected in the fatty acid profile of the plant. In addition, the *KTi*3 was silenced, which is explained by the fact that the *KTi*3 promoter was used to drive the expression of the *gm-fad2-1* gene fragment.

The levels of GM-HRA were analysed at six locations in the USA and in Canada (2005 growing season) and at six locations in Chile and Argentina (2005–2006 growing season) by a specific enzyme-linked immunosorbent assay (ELISA) system developed for this protein. The mean level of the protein ranged from 2.1 to 2.5 µg/g seed dry weight and was not affected by spraying the plants with ALS-inhibiting herbicides. The protein was also present in the leaf, forage and root.

3.1.4. Inheritance and stability of the inserted DNA[11]

The inserted DNA in soybean 305423 is integrated in the soybean nuclear genome. Genetic stability was evaluated by studying the inheritance and segregation pattern of the introduced genetic material in several generations. Southern analysis using one restriction enzyme and *gm*-fad2-1 and *gm-hra* probes was performed on plants of the T_4, T_5 and F_2 generations (T_3 generation crossed to elite lines and selfed once). Stability within a single generation was studied using 100 individuals of the F_2 generation which were analysed for oleic acid content and by soybean 305423 event-specific PCR. One plant had undergone recombination between two repeated *KTi*3 promoter elements within insertion region 1, removing the entire *gm-hra* cassette and portions of the *KTi*3 promoter elements flanking the cassette. Subsequent studies with more than 1 000 individuals did not identify any recombination events. It may be that insertion region 1 can be more prone to instability owing to the possibility of recombination between the two repeated *KTi*3 promoter elements. However, loss of part of region 1 does not raise any safety issue.

The inserted DNA is genetically linked and segregates following a typical pattern of Mendelian inheritance expected for a single, genetically linked insertion locus. Analysis of the GM-HRA protein and fatty acid profile of seeds collected across several locations in Chile, Argentina, the USA and Canada confirmed the phenotypic stability of the introduced traits.

3.2. Conclusion

The molecular characterisation data establish that genetically modified (GM) soybean 305423 contains four complete and partial copies of the *gm-fad2-1* and *gm-hra* expression cassettes. No other parts of the plasmid used for transformation are present in the transformed plant except for a small, non-functional vector fragment which does not include the origin of replication or the hygromycin resistance gene. Results of updated bioinformatic analyses of the 5′ and 3′ flanking sequences and ORFs present in the junction regions and within the insert did not indicate a safety issue. One plant out of the 1 100 tested individuals had undergone recombination and, as a result, had lost the entire *gm-hra* cassette and portions of the promoter elements flanking the cassette. However, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) is of the opinion that this recombination, leading to loss of the trait, raises no safety issues. The stability of the inserted DNA was sufficiently confirmed over several generations. The four insertions are genetically linked and behave as a single Mendelian locus.

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 GMO Panel, 2010a, 2011a). This recommends the simultaneous application of a test of difference to determine whether the GM plant is different from its

[10] Technical dossier/Section D3.
[11] Technical dossier/Section D5.

conventional counterpart, and a test of equivalence to determine whether the GM plant falls within the 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[12]

The applicant provided agronomic and compositional data from field trials which used negative segregants as the only comparators. These field trials were performed at six locations in the USA and Canada in 2005 and at six locations during the season 2005–2006 in Chile and Argentina. As negative segregants are derived from a GM organism, the GMO Panel does not consider them as appropriate comparators with a history of safe use (EFSA, 2011a).

On request of the EFSA GMO Panel, the applicant provided data for agronomic and phenotypic and compositional analyses where soybean 305423 was compared to the non-GM variety Jack, which is an appropriate conventional counterpart with a history of safe use. These additional data were from field trials carried out in the seasons 2005, 2010 and 2011 in North America.[13] The field trial in 2005 did not include GM soybean plants treated with the intended herbicide. The field trial carried out in 2010 lacked sufficient statistical power because of insufficient replications. The 2011 field trial was performed at ten sites within soybean cultivation areas in the USA. At each site, the following test materials were grown in a randomised complete block design with four replicates: soybean 305423 treated and untreated with the intended herbicide, the conventional counterpart Jack and non-GM reference varieties (10 across all sites).[14] The EFSA GMO Panel considers that the 2011 field trial was performed in accordance with the most recent Guidance document (EFSA, 2011a) and data from this field trial formed the basis for the further assessment.

4.1.2. Agronomic and phenotypic characteristics[15]

Based on data collected at the ten sites in the USA field trial in 2011 (the same field trial used to collect seeds and forage for compositional studies, see sections 4.1.1 and 4.1.3), the applicant performed a comparative assessment of the agronomic and phenotypic characteristics of soybean 305423 and its conventional counterpart, as well as between soybean 305423 and the non-GM reference soybean varieties grown in the same sites. The ten agronomic and phenotypic characteristics evaluated were: early population, final population, seedling vigour, plant height, disease incidence, insect damage, lodging, shattering, days to maturity and yield.

The test of difference of the agronomic and phenotypic characteristics of soybean 305423 (either sprayed with maintenance pesticides or sprayed with the intended herbicide on top of the maintenance pesticide) compared with the conventional counterpart identified statistically significant differences for six endpoints (early population, final population, seedling vigour, plant height, shattering and yield). The equivalence test indicated that all the characteristics analysed (except for shattering[16]) fell within the equivalence limits established from the non-GM soybean reference varieties. Considering these outcomes of the equivalence test and the type of characteristics, the GMO Panel found that the identified differences are not an indication for an unintended effect that would significantly impact on crop biology.

[12] Technical Dossier/Sections D7.2 and D7.3 and additional information, March 2012.
[13] Additional information, February 2008 and 2011 and March 2012.
[14] Pioneer® Brand Soybean Line 92M10, Pioneer® Brand Soybean Line 92M22, Pioneer® Brand Soybean Line 92M72, Pioneer® Brand Soybean Line 92Y21, Pioneer® Brand Soybean Line 93B82, Pioneer® Brand Soybean Line 93M14, Pioneer® Brand Soybean Line 93M5, Pioneer® Brand Soybean Line 93M62, Pioneer® Brand Soybean Line 93Y21, Pioneer® Brand Soybean Line 93Y41.
[15] Technical Dossier/Section D4 and additional information, January 2012.
[16] The test of equivalence could not be performed on shattering because of the lack of variation among the non-GM reference varieties for this endpoint.

4.1.3. Compositional analysis[17]

The key constituents included in the compositional analysis of soybean seeds and forage were in accordance with Organisation for Economic Co-operation and Development (OECD) recommendations (OECD, 2001). Specific fatty acids were included because of the nature of the genetic modification. In total, 92 endpoints were measured in seeds and seven were measured in forage. Nineteen parameters having more than 50 % of the observations below the limit of quantification were excluded from the comparative analysis. In total, 73 parameters were statistically analysed in seeds and seven were analysed in forage.[18]

Plants sprayed with maintenance pesticides showed statistically significant differences between soybean 305423 and its conventional counterpart for 51 parameters in seeds and four parameters in forage (crude fat, crude fibre, neutral detergent fibre and acid detergent fibre). For differences observed in 34 out of the 51 seed parameters, no further assessment was required, because they fell within the equivalence limits established from the non-GM soybean reference varieties included in the study. Equivalence could not be established for 16 of the 51 seeds' parameters (equivalence category III or IV). These compounds were myristic acid, palmitic acid, heptadecanoic acid, heptadecenoic acid, oleic acid, linoleic acid, linolenic acid, isomer 2 of nonadecenoic acid, arachidic acid, eicosenoic acid, behenic acid, lignoceric acid, zinc, glycitin, total glycitein equivalents and trypsin inhibitor (Table 1). The test of equivalence could not be performed on one of the 51 seeds' parameters showing statistically significant differences, namely (9,15) isomer of linoleic acid (C18:2), because of the lack of variation among the non-GM soybean reference varieties for this compound. Equivalence could not be established for two forage parameters (neutral detergent fibre and acid detergent fibre) that showed significant differences (equivalence category III) (Table 1). The test of equivalence could not be performed on one forage parameter showing a significant difference (crude fibre) because of the lack of variation among the non-GM soybean reference varieties for this compound.

Samples sprayed with the intended herbicide in addition to the maintenance herbicide showed statistically significant differences between soybean 305423 and its conventional counterpart (sprayed with the maintenance pesticides) for 53 compounds in seeds and three compounds in forage (crude fibre, neutral detergent fibre and acid detergent fibre). The test of equivalence indicated that 36 of the 53 parameters in seeds fell within the equivalence limits established from the non-GM soybean reference varieties. Equivalence could not be established for 16 of the 53 seeds' parameters (equivalence categories III and IV). These compounds were myristic acid, palmitic acid, heptadecanoic acid, heptadecenoic acid, oleic acid, linoleic acid, linolenic acid, isomer 2 of nonadecenoic acid, arachidic acid, eicosenoic acid, behenic acid, lignoceric acid, glycitin, total glycitein equivalents, trypsin inhibitor and calcium (Table 1). The test of equivalence could not be performed on one of the 53 statistically significantly different seeds' parameters (acid detergent fibre) because of the lack of variation among the non-GM soybean reference varieties for this compound. Equivalence could not be established for one of the three forage parameters showing significant

[17] Technical Dossier/Sections D7.2 and D7.3 and additional information, March 2012.

[18] Compounds having quantifiable levels in seeds were: proximates and fibre fractions (crude protein, crude fat, crude fibre, acid detergent fibre, neutral detergent fibre, ash, carbohydrates (by calculation)), fatty acid profile (myristic acid, palmitic acid, palmitoleic acid, heptadecanoic acid, heptadecenoic acid, stearic acid, oleic acid, linoleic acid, (9,15) isomer of linoleic acid, linolenic acid, isomer 2 of nonadecenoic acid, arachidic acid, eicosenoic acid, behenic acid, tricosanoic acid, lignoceric acid), total amino acids (alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, zinc), vitamins (vitamin B1, vitamin B2, vitamin B3, vitamin B5, vitamin B6, vitamin B9, α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol, total tocopherols), antinutrients and other secondary compounds (daidzein, daidzin, genistin, glycitin, total daidzein equivalents (by calculation), total genistein equivalents (by calculation), total glycitein equivalents (by calculation), raffinose, stachyose, sucrose, lectins, phytic acid and trypsin inhibitor). Compounds analysed in forage were proximates and fibre fractions (crude protein, crude fat, crude fibre, acid detergent fibre, neutral detergent fibre, ash and carbohydrates (by calculation)). Compounds where 50 % or more sample values were below the lower limit of quantification in seed were: caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristoleic acid (C14:1), pentadecanoic acid (C15:0), pentadecenoic acid (C15:1), heptadecadienoic acid (C17:2), γ-linolenic acid (C18:3), nonadecanoic acid (C19:0), isomer 1 of nonadecenoic acid (C19:1), eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3), arachidonic acid (C20:4), heneicosanoic acid (C21:0), erucic acid (C22:1), sodium, genistein, glycitein, coumestrol.

differences (neutral detergent fibre; equivalence category III). The test of equivalence could not be performed on one forage parameter showing a significant difference (crude fibre) because of the lack of variation among the non-GM soybean reference varieties for this compound (Table 1).

Table 1 shows the mean values for the endpoints which were statistically different and fell into equivalence categories III and IV. The observed differences of the fatty acid profile are consistent with the intended effect of the genetic modification, i.e. an increase in oleic acid at the expense of PUFA. Changes in the levels of odd chain fatty acids are an unintended effect probably caused by the introduction of the ALS enzyme. An explanation provided by the applicant, which is considered plausible by the EFSA GMO Panel, is that the newly introduced GM-HRA enzyme, acting in a similar way to ALS to confer herbicide tolerance, may have a decreased affinity for 2-ketobutyrate owing to the replacement of tryptophan 560 by leucine. The decreased affinity of the GM-HRA enzyme for 2-ketobutyrate may lead to higher concentrations of 2-ketobutyrate in soybean 305423. As the odd chain fatty acid biosynthesis starts with the conversion of 2-ketobutyrate to propionyl-CoA, followed by the subsequent addition of C2 moieties, an increased pool of 2-ketobutyrate available for odd chain fatty acid biosynthesis may lead to increased levels of such fatty acids. The changed fatty acid profile is assessed for possible nutritional and safety implications in section 5.

The other parameters (calcium, zinc and glycitin and related total glycitein equivalents) showing non-equivalence were further evaluated by the EFSA GMO Panel. The Panel took into account their well-known biochemical roles and the magnitude of the reported levels and concluded that the reported levels lack relevance from a food and feed safety and nutritional point of view. The EFSA GMO Panel noted that the variation for glycitin in soybean exceeds the lower and upper limits established by the non-GM reference varieties growing in the same field trial (Al-Tawaha et al., 2007; Gutierrez-Gonzalez et al., 2009).

The lack of equivalence for trypsin inhibitor was the result of a decrease of its level in soybean 305423. This is in line with the Northern analysis results indicating silencing of the trypsin inhibitor gene transcription in soybean 305423 seed. A decreased level of trypsin inhibitor is not posing any safety concern.

For the differences in forage fibre fractions for which equivalence could not be established or the equivalence test could not be performed, the EFSA GMO Panel considered that, based on the well-known biochemical properties of fibre fractions and the magnitude of levels in forage, these endpoints do not require further assessment.

Table 1: Levels of constituents in forage and seeds harvested from field trials with soybean 305423 and the conventional counterpart Jack, which were statistically significantly different between 305423 and Jack and which fell into equivalence categories III and IV

Constituents	Means and standard deviations (in brackets) across locations (2011 field trial)		
	Conventional counterpart (Jack)	Soybean 305423 (untreated)	Soybean 305423 (treated)
Equivalence category IV			
Saturated fatty acids [(a)]			
Myristic acid (C14:0)	0.07 (0.012)	0.04 (0.010)	0.04 (0.010)
Palmitic acid (C16:0)	10.1 (0.283)	6.48 (0.295)	6.50 (0.334)
Heptadecanoic acid (C17:0)	0.11 (0.036)	0.81 (0.013)	0.81 (0.054)
Arachidic acid (C20:0)	0.32 (0.029)	0.42 (0.029)	0.42 (0.028)
Behenic acid (C22:0)	0.33 (0.038)	0.43 (0.025)	0.43 (0.034)
Tricosanoic acid (C23:0)	0.053 (0.012)	0.064 (0.010)	0.065 (0.014)
Lignoceric acid (C24:0)	0.14 (0.036)	0.20 (0.024)	0.19 (0.035)
Mono-unsaturated fatty acids [(a)]			
Heptadecenoic acid (C17:1)	0.06 (0.036)	1.26 (0.013)	1.26 (0.013)
Oleic acid (C18:1)	19.2 (2.531)	73.7 (1.836)	72.7 (4.626)
Isomer 2 nonadecenoic acid (C19:1) [(b)]	0.04 (0.038)	0.31 (0.027)	0.32 (0.040)
Eicosenoic acid (C20:1)	0.17 (0.046)	0.35 (0.023)	0.35 (0.046)
Polyunsaturated fatty acids [(a)]			
Linoleic acid (C18:2)	54.9 (1.723)	4.26 (1.619)	4.48 (2.753)
Linolenic acid (C18:3)	8.28 (0.950)	4.84 (0.939)	4.72 (1.062)
Other constituents			
Zinc [% DW]	0.005 (0.001)	0.006 (0.001)	0.005 (0.001)
Trypsin inhibitor [IU/mg DW]	29.4 (2.964)	13.8 (5.038)	14.6 (2.009)
Equivalence category III			
Other constituents			
Acid detergent fibre [% DW][19]	33.7 (2.031)	31.5 (1.424)	31.6 (2.092)
Neutral detergent fibre [% DW][19]	44.0 (1.079)	41.3 (1.458)	41.6 (0.889)
Calcium [% DW]	0.26 (0.031)	0.24 (0.046)	0.23 (0.027)
Glycitin [mg/kg DW]	310 (28.949)	423 (20.309)	418 (35.112)
Total Glycitein [mg/kg DW]	200 (18.542)	272 (12.752)	270 (21.417)

(a): Fatty acid proportions are given as percentages of total fatty acids. Soybean 305423 was either treated with intended herbicide (treated) or treated with conventional herbicide (untreated).
(b): Position of the double bond in nonadecenoic acid not known.

4.2. Conclusion

The EFSA GMO Panel concludes that the composition of soybean 305423 differs from that of the conventional counterpart and of non-GM reference varieties in its fatty acid profile, the newly

[19] Parameter analysed in forage.

expressed protein, the minerals zinc and calcium and the isoflavone glycitin. The variations in the fatty acid profile and the newly expressed protein are consistent with the objective of the modification as well as with the expression of the ALS enzyme of soybean 305423. A safety and nutritional assessment of the altered fatty acid profile and the newly expressed protein is provided in section 5 of this Scientific Opinion. For the remaining compounds, no further assessment was deemed necessary owing to their well-known biochemical roles and to the magnitude of the reported levels.

The EFSA GMO Panel also concludes that no differences were identified in the agronomic and phenotypic characteristics that would require further assessment with regard to safety.

5. Food/feed safety assessment

5.1. Evaluation of relevant scientific data

5.1.1. Effects of processing[20]

No novel method of production and processing is envisaged.

The applicant studied the influence of temperature (36–60 °C) and pH value (5.0–9.0) on the enzyme activity of the GM-HRA protein produced in *Escherichia coli* (see section 5.1.2.1) using an ALS activity assay based on the production of acetolactate from pyruvate. After incubation at 44 °C for 15 minutes, approximately 50 % of the activity was lost, and the enzyme was inactivated after incubation at 50 °C for 15 minutes. The optimal pH for the enzyme activity was in the range pH 7.0–7.5, whereas there was practically no activity at or below pH 6.0 as well as at pH 9.0.

5.1.2. Toxicology[21]

5.1.2.1. Protein used for safety assessment[22]

The only newly expressed protein in soybean 305423 is the GM-HRA protein. Given its low levels of expression in soybean 305423, GM-HRA protein produced in a recombinant *E. coli* strain (BL21 (DE3)RIPL) was used for the safety assessment.

The mature form of the GM-HRA protein, not containing the N-terminal chloroplast transit peptide cleaved from the protein during processing in the plant, was produced in *E. coli* in the form of a fusion protein. The purification process included the cleavage of the His-tag with thrombin; the resulting microbial GM-HRA protein has an additional glycine residue at the N-terminus (resulting in a total of 604 amino acids) compared with the mature GM-HRA protein expressed in soybean 305423 leaves. The equivalence of the GM-HRA protein produced in *E. coli* to that produced in leaf tissue of soybean 305423 was shown by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and protein staining, Western analysis, N-terminal amino acid sequence analysis, matrix-assisted laser desorption/ionisation mass spectrometry (MALDI-MS) analysis of tryptic peptides and glycosylation analysis. In addition, the identity of the microbial protein was corroborated using electrospray ionisation mass spectroscopy, analysis of the amino acid composition and determination of the enzyme activity.

Based on the identified similarity in structure and equivalence in physico-chemical properties and function between microbial and soybean 305423 GM-HRA proteins, the EFSA GMO Panel accepts the use of GM-HRA produced in *E. coli* as an appropriate substitute test material for the GM-HRA protein present in soybean 305423.

[20] Technical Dossier/Section D7.6.
[21] Technical Dossier/Section D7.8 and additional information, October 2010 and April 2011.
[22] Technical Dossier/Section D7.8.1.

5.1.2.2. Toxicological assessment of the expressed novel protein in soybean 305423[23]

The GM-HRA protein expressed in soybean 305423 is an ALS encoded by a modified *als* gene from soybean (*Glycine max*).

The EFSA GMO Panel has previously evaluated the safety of the GM-HRA protein in the context of application EFSA-GMO-UK-2007-43, which included an acute and a 28-day repeated-dose toxicity study in mice and *in vitro* pepsin- and pancreatin-resistance tests. No safety concerns were identified (EFSA, 2011b).

The bioinformatics-supported comparison of the amino acid sequence of the GM-HRA precursor protein expressed in soybean 305423 has been updated (February 2013) and revealed no significant similarities to known toxic proteins.[24]

5.1.3. Animal studies with the food/feed derived from soybean 305423

The applicant performed a 95-day feeding study in rats, a 42-day study in chickens for fattening, a 12-week study in laying hens and a 77-day study in pigs using diets containing processed fractions of soybean 305423 or control soybean. Dehulled, fat-extracted toasted soybean meal was the principal product tested, with small amounts of hulls and/or oil added in the rat and chicken studies. In the rat and chicken studies, the applicant used as a control soybean products from a negative segregant. Additional groups were fed with commercial non-GM soybean varieties.[25]

5.1.3.1. Sub-chronic toxicity study in rats[26]

Groups of 12 male and 12 female Crl:CD(SD) rats, individually housed, were *ad libitum* fed balanced rodent diets for 95 days incorporating dehulled fat-extracted toasted soybean meal (19.8–20 %), toasted ground hulls (1.43–1.5 %) and degummed alkaline-refined oil (1.53–1.73 %) derived, respectively, from soybean 305423 (test group, verified by PCR), a negative segregant (control group); and commercial non-GM soybean varieties (93B86, 93B15 or 93M40). Soybean 305423 was not treated with the intended herbicide.[27] All soybean fractions and diets were nutritionally similar, with the expected fatty acids level changes in 305423 oil and diet. Effects of 305423 diet on standard endpoints (OECD 408) were compared by analysis of variance (ANOVA) with those from control. Data from animals fed commercial varieties were used to obtain information on the normal range of the examined parameters.

There were no deaths during the study. Regular observations of the animals did not reveal clinically relevant effects. Apart from some isolated statistically significant differences between the test and control group, body weight, body weight gain, feed consumption and feed efficiency were similar across all groups. Ophthalmological as well as neurobehavioral evaluations (an abbreviated functional observational battery and motor activity measurements) showed no relevant differences between groups. Haematology, coagulation and clinical chemistry analyses showed no statistically significant differences between the test and control group. Urinalysis findings were unremarkable. A statistically significant lower heart weight (relative to brain weight) was seen in males fed the test diet in comparison with controls. This was not accompanied by changes in other cardiac endpoints (absolute and relative-to-body organ weight; macroscopic or microscopic findings) and is considered incidental. No relevant differences between groups were seen at macroscopic examination. Microscopic examination was only performed in the test and control group, and the nature and the incidence of the findings were similar and typical for animals of this strain and age.

The EFSA GMO Panel considers that a repeated-dose 90-day oral toxicity study, in which material derived from a negative segregant is administered as the sole control material, has limitations for the

[23] Technical dossier/Section D1.
[24] Technical dossier/Section D7.8 and additional information, May 2013.
[25] Additional information, September 2010.
[26] Additional information, May 2008/Annex 2, and Delaney et al., 2008.
[27] Additional information, September 2010/Annex 6.

safety assessment, principally because of an inability to detect unintended effects. An appropriate non-GM genotype with a genetic background as close as possible to soybean 305423 with a history of safe use (conventional counterpart) should have been included in this study. However, three commercial non-GM varieties were included providing information on the normal range. The values and/or nature of the findings were comparable throughout all groups.

5.1.3.2. Chicken feeding study[28]

The applicant provided a 42-day study, which was also described in a scientific publication (McNaughton et al., 2008). Six hundred commercial Ross × Cobb broilers were assigned to five experimental groups (n = 60/sex/group, housed five per sex and per pen for a total of 12 pens per group) fed diets containing processed products, respectively, from soybean 305423 (test group), the negative segregant (control group) or commercial non-GM varieties (993B86, 93B15 and 93M40). Soybean 305423 was not treated with the intended herbicide.[29] Chickens were offered *ad libitum* starter (days 0 to 21), grower (days 22 to 35) and finisher diets (days 36 to 42) with, respectively, 26.5 %, 23 % or 21.5 % toasted soybean meal and fixed amount of soybean hulls and oil (1 % or 0.5 %, respectively); hulls and oil were in a lower proportion than in conventional formulations owing to their different nutritional values in comparison with the typical commercial counterparts (higher hull nutritional values and lower oil gross energy values). Before mixing the feed, meals and hulls were analysed for proximates and minerals, amino acids, mycotoxins and oils for fatty acid composition. Diets were adjusted according to the National Research Council (NRC) Nutrient Requirement for Poultry (1994) to be isonitrogenous, isocaloric and balanced for minerals, sulphur and limiting amino acids (analytically confirmed). PCR confirmed the presence of the DP-305423-1 event in the soybean 305423 diet only.[30] The GM-HRA protein was below the low limit of quantification (< 0.27 ng/mg).

Effects of soybean 305423 diet on health status, survival, body weight and feed intake (taken at weekly intervals), body weight gain and feed efficiency (calculated from day 0 to day 42), terminal carcass and carcass parts weight from four chicken/sex/pen (thighs, breasts, wings, legs, abdominal fat; kidneys and liver as indicators of health from dietary inadequacies) were compared with those from control. Statistical analysis was performed by a mixed ANOVA using the pen as the experimental unit for health status, survival, body weight, body weight gain, feed intake, feed efficiency and terminal live weight without considering sex, using instead individuals for carcass data, taking into account the sex. For statistically significant differences ($P \leq 0.05$) the Benjamin–Hochberg analysis for false discovery rate was applied. Data from animals fed commercial varieties were used to obtain 95 % tolerance ranges.

An overall low mortality was observed (0.83–1.67 %), with equal values for controls and test diet groups (1/120). Final body weights and body weight gain (day 0–42) from test diet fed chicken were slightly lower in comparison with concurrent controls (body weight: 1862.3 g test diet, 1905.5 g control diet; body weight gain: 1814.4 g test diet, 1857.4 g control diet); however, these differences were not statistically significant and within the 95 % tolerance range derived from the additional groups fed commercial non-GM varieties. Significant differences in feed per gain ratio (1.87 test diet, 1.86 control diet) or in any carcass yield data were not obtained.

The EFSA GMO Panel considers that a 42-day feeding study in broilers, in which material derived from a negative segregant is administered as the sole control material, has limitations for the nutritional assessment, principally because of an inability to detect unintended effects. The use of low energy diets (about 3–3.5 % fat) further reduced the capacity of the study to detect unintended effects. As the diets were formulated to provide the same nutrition, the expectation was that chicken from the

[28] Technical dossier/Annex 9 and additional information, October 2012.
[29] Additional information, May 2013/Annex 6.
[30] A low-level contamination by MON-04032-6 event was detected in six out of twelve determinations of the grower soybean 305423 test diet. The MON-04032-6 event is associated with the expression of cp4 epsps protein which confers herbicide tolerance via a different mechanism of action. It is therefore considered that any influence on the zootechnical results is unlikely.

five experimental groups would show essentially the same performance characteristics. Results confirmed the nutritional value of soybean toasted defatted meal derived from soybean 305423, as the zootechnical performance parameters of the test group were within the 95 % tolerance intervals derived from the three commercial non-GM varieties.

5.1.3.3. Laying hen feeding study[31]

A 12-week feeding study (composed of three 4-week phases according to egg production stage) was conducted on Hy-Line W-36 Single Comb White Leghorn hens (Mejia et al., 2010). Pullets ($n = 336$, 20 weeks old) were placed in 24 experimental units (cage lots, with two cages/lot, seven hens/cage), which were randomly assigned to four groups (six cage lots/group). After a 5-week adaptation period, hens were fed diets containing 22.95–23.87 % dehulled fat-extracted toasted soybean meals from 305423 (test group, PCR confirmed), the conventional counterpart Jack (control group) and commercial non-GM varieties (92M72 and 93B15). Compositional data were provided only for the soybean meals used in the study. Only soybean 305423 was treated with the intended herbicide.[32] Balanced diets were formulated according to NRC (1994) to be isonitrogenous and isocaloric (GE), with further adjustments for sulphur amino acids, lysine, threonine, tryptophan and arginine (analytically confirmed). Commercial soybean oil (1.2 %) was added to all diets.

Zootechnical performance and egg quality parameters were recorded and calculated on a phase basis. The comparison between soybean 305423 and control groups was conducted on summarised overall study data. Statistical analysis was based on a mixed model with treatment, phase and treatment × phase interaction as fixed effects and cage lot as a random effect. For statistically significant differences ($P \leq 0.05$) the Benjamin–Hochberg analysis for false discovery rate was applied. Data from commercial non-GM varieties fed animals were used to obtain 95 % tolerance intervals.

Sporadic mortalities occurred in the groups fed with soybean 305423, control and 92M7 groups (one hen/group). Apart from an incidental decline in production (egg/hen/day and egg mass) seen in one cage lot fed soybean 305423 diet during the second and third study phase owing to a few individuals going out of production, no significant differences in performance and egg quality parameters were seen between hens fed the 305423 and the control diets.

As the diets were formulated to provide the same nutrition, the expectation was that hens from the four experimental groups would show essentially the same performance characteristics. Results confirmed the nutritional value of soybean toasted defatted meal and the absence of any unintended effects impacting on performance at the tested level.

5.1.3.4. Pig feeding study[33]

In a 77-day feeding study (Miller et al., 2011), 64 individually housed pigs, 27 kg weight, were randomly allocated to four groups (eight animals/sex/group). The experiment took place in two rooms of a single environmentally controlled barn, with treatment replicates blocked by room (four animals/sex/group/room). This study followed a three-phase programme (grower phase, days 1–34; early finisher phase, days 35–62; and late finisher phase, days 63–77). The grower, early finisher and late finisher diets, formulated and balanced (analytically confirmed) according NRC (1998), contained 24.23–26.44 %, 19.58–20.71 % or 14.95–16.27 % soybean meal, respectively, and were offered in mash form *ad libitum*. The four experimental groups received diets with 305423 (test diet, PCR confirmed), conventional counterpart Jack (control diet) and two non-GM commercial soybean

[31] Additional information, May 2013, and Mejia et al., 2010.
[32] Additional information, May 2013/Annex 7.
[33] Additional information, May 2013, and Miller et al., 2011.

varieties (92M72; 93B15). Compositional data were provided for only the soybean meals used in the study. Only soybean 305423 was treated with the intended herbicide.[34,35]

Pigs were weighed at the beginning of the experiment and on days 14, 35, 49, 63 and 77. Feed allocation was recorded daily. Comparison between test diet- and control diet-fed animals was performed on zootechnical performance measures (final body weight, average daily gain, average feed daily intake and gain-to-feed ratio) and on carcass data (dressing percentage, hot weight, tenth rib backfat thickness, loin eye area, lean meat percentages, loin depth measured at the tenth rib). The individual pig was the experimental unit for the data analysis, which was conducted on sex-combined data. A mixed ANOVA model was used for the analysis, with treatment, sex and treatment × sex interaction as fixed effects and room assignment as a random effect in the analysis of growth and carcass data (also harvest day as a random effect). For statistically significant differences ($P \leq 0.05$) the Benjamin–Hochberg analysis for false discovery rate was applied. Data from animals fed the additional commercial varieties were used to obtain 95 % tolerance intervals.

Two incidental barrow mortalities occurred (diagnosed as *Salmonella* infection-related, one control from one room, and one test diet barrow from the other room) and one control female showed poor feed intake and weight loss throughout the study, despite antibiotic treatment, and were eventually dropped from the statistical analysis. No significant differences (false discovery rate adjusted $P < 0.05$) were found for all endpoints examined.

As the diets were formulated to provide the same nutrition, the expectation was that pigs from the four experimental groups would show essentially the same performance characteristics. Results confirmed the nutritional value of soybean toasted fat-extracted meal and the absence of any unintended effects impacting on performance at the tested level.

5.1.4. Allergenicity[36]

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

5.1.4.1. Assessment of allergenicity of the newly expressed protein[37]

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

The EFSA GMO Panel has previously evaluated the safety of the GM-HRA protein in the context of application EFSA-GMO-UK-2007-43 and no concerns on allergenicity were identified (EFSA, 2011a).

Updated bioinformatic analyses[38] of the amino acid sequence of the GM-HRA precursor protein using the criterion of 35 % identity in a window of 80 amino acids revealed no significant similarities to known allergens. In addition, the applicant performed analyses[39] searching for matches of eight contiguous identical amino acid sequences between the GM-HRA precursor protein and known allergens, which confirmed the outcome of the previous bioinformatic analyses.

[34] Additional information, May 2013/Annex 6.
[35] Additional information, May 2013/Annex 7.
[36] Technical dossier/Section D7.9 and additional information, October 2010.
[37] Technical dossier/Section D7.9.1 and additional information, May 2013.
[38] Additional information, May 2013.
[39] Additional information, May 2013.

Based on all the information available, the EFSA GMO Panel considers that there are no indications that the protein GM-HRA present in soybean 305423 may be allergenic in the intended conditions of use.

5.1.4.2. Assessment of allergenicity of the whole GM plant or crop

Allergenicity of the whole GM plant could be increased as an unintended effect owing to the genetic modification, for example through qualitative or quantitative modifications of the pattern of expression of endogenous proteins.

According to the EFSA GMO Panel Guidance document (EFSA, 2006a), when the plant receiving the introduced gene is known to be allergenic, the applicant should test any potential change in the allergenicity of the whole GM plant by comparing the allergen repertoire with that of its appropriate conventional counterpart(s). In this context, soybean is also considered a common allergenic food (EC, 2007).

Initially, the applicant performed *in vitro* allergenicity studies with extracts of seeds from soybean 305423 and its conventional counterpart. The applicant provided one-dimensional (1-D) immunoglobulin (IgE) immunoblot analysis as well as ELISA inhibition tests using pooled sera from individuals allergic to soybean. Based on these data only, the EFSA GMO Panel could not conclude on the endogenous allergenicity of soybean 305423 owing to the limitations associated with these two methodologies (see Annex 4 and Annex 5 of EFSA GMO Panel, 2010b).

At the request of the EFSA GMO Panel, the applicant provided additional information (2-D immunoblot and ELISA analyses) using individual sera from eight subjects with clinically confirmed allergy to soybeans and five negative control sera. In the 2-D immunoblot analysis, no meaningful differences in the IgE binding patterns were detected between extracts of soybean 305423 and its conventional counterpart. In the ELISA analysis, the sera from allergic individuals had similar reactivity to proteins in extracts from soybean 305423 and the conventional counterpart.

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 305423 when compared with that of its conventional counterpart.

5.1.5. Nutritional assessment of GM food/feed

5.1.5.1. Human exposure

The main product for human consumption is the oil of soybean 305423, which has a high content of oleic acid and a reduced content of PUFA. It is intended for use in commercial frying and spraying only.[40] Concerning this use, the applicant provided three different exposure scenarios exclusively for fried foods.[41] Subsequently, the applicant provided a fourth scenario covering the use of this oil in targeted foods and other foods.[42] The Panel has considered the last scenario only, because it covers the greatest number of food items, and it is therefore more conservative.

Consumption data are taken from the UK National Diet and Nutrition Survey (NDNS) of 2008–2010 (Bates et al., 2011). The sub-populations considered are toddlers (1–3 years), children (4–10 years), teenagers (11–18 years), adults (19–64 years) and the elderly (≥ 65 years). The content of a specific vegetable oil in foods was calculated. Food items considered are the targeted foods (fried fish, meat, potatoes, vegetables and other fried foods, home-use; and from spray applications savoury snacks and crackers) and other foods (salad dressings, margarines and spread, mayonnaise).

[40] Technical dossier/Section D7.10.1 and additional information, November 2008.
[41] Technical dossier/Annex 15, additional information, November 2008, and additional information, May 2013.
[42] Additional information spontaneously provided in October 2013.

The fatty acid composition of the oil from soybean 305423 is taken from that of the unprocessed seeds from the field trial of 2011.[43] The oil is assumed to fully replace vegetable oils in the individual food items.[44] Vegetable oils considered were rapeseed oil, sunflower oil, palm oil and blends of these oils. Whenever the type of vegetable oil could not be identified, composite oil with an equal blend of high-oleic sunflower, palm and canola oil was assumed.

This enabled an assessment of estimated daily intakes from both domestically and commercially prepared foods. Results are given as means and expressed as g/day (Table 2) and as percentage of energy (E %) of the total diet (Table 3) for five fatty acid groups (saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), n-6 PUFA, n-3 PUFA and trans fatty acids (TFA)). Average and upper percentile intake amounts of the relevant food groups were calculated.

Table 2: Estimated daily intake (g) of fatty acid groups before (B) and after (A) the replacement of the vegetable oils with the soybean 305423 oil

Consumer group [(a)]	Mean SFA		Mean MUFA		Mean n-6 PUFA		Mean n-3 PUFA		Mean TFA	
	B	A	B	A	B	A	B	A	B	A
Males										
Toddlers	19.3	18.9	14.7	18.3	5.0	3.7	0.9	1.4	0.9	0.9
Children	24.0	23.4	21.1	28.2	7.7	5.6	1.4	2.4	1.3	1.2
Teenagers	28.3	27.9	27.9	38.0	10.4	7.4	2.0	3.9	1.6	1.5
Adults	29.7	29.1	29.2	37.5	11.7	8.2	2.4	3.2	1.8	1.7
Elderly	30.4	29.7	26.3	31.8	10.6	7.4	2.4	2.8	1.9	1.8
Females										
Toddlers	18.1	17.7	14.0	18.1	5.1	3.5	0.9	1.6	0.9	0.8
Children	22.8	22.3	20.8	27.8	7.8	5.5	1.5	2.6	1.3	1.2
Teenagers	22.9	22.5	23.6	34.1	9.0	6.0	1.8	3.6	1.4	1.3
Adults	22.4	22.0	21.6	27.6	9.0	6.4	1.9	2.6	1.3	1.3
Elderly	24.0	23.6	19.9	23.8	8.0	5.9	1.9	2.2	1.4	1.4

(a): Consumer group by age: toddlers (1–3 years), children (4–10 years), teenagers (11–18 years), adults (19–64 years) and the elderly (≥ 65 years).

Table 3: Estimated daily intake (E %) of fatty acid groups before (B) and after (A) the replacement of the vegetable oils with the soybean 305423 oil

Consumer group [(a)]	Mean SFA		Mean MUFA		Mean n-6 PUFA		Mean n-3 PUFA		Mean TFA	
	B	A	B	A	B	A	B	A	B	A
Males										
Toddlers	14.7	14.7	11.3	14.6	3.9	3.2	0.7	1.2	0.7	0.7
Children	13.4	13.5	11.9	16.7	4.4	3.6	0.8	1.5	0.7	0.7
Teenagers	12.5	12.6	12.5	17.7	4.8	3.8	0.9	1.8	0.7	0.7
Adults	12.1	12.2	11.9	16.1	4.8	3.9	1.0	1.4	0.7	0.7
Elderly	13.8	14.0	11.9	15.3	4.8	4.0	1.1	1.4	0.9	0.8
Females										
Toddlers	14.8	14.8	11.4	15.2	4.1	3.2	0.7	1.4	0.7	0.7
Children	13.3	13.4	12.2	17.2	4.6	3.7	0.9	1.6	0.7	0.7
Teenagers	12.4	12.6	12.9	19.4	4.9	3.7	1.0	2.0	0.8	0.7
Adults	12.1	12.2	11.6	15.7	4.9	4.0	1.0	1.5	0.7	0.7
Elderly	14.0	14.2	11.7	14.7	4.8	4.0	1.1	1.3	0.8	0.8

(a): Consumer group by age: toddlers (1–3 years), children (4–10 years), teenagers (11–18 years), adults (19–64 years) and the elderly (≥ 65 years).

[43] Additional information, June 2013.
[44] The applicant also considered two other scenarios with lower substitution rates (50 % and 20 %).

EFSA has not set a dietary reference value for SFA. Several international and national authorities on nutrition recommend SFA intakes of < 10 E % (see EFSA NDA Panel, 2010). The baseline SFA intake in all population groups (see Table 2) is higher than 10 E %, but does not change through the replacement of vegetable oils with soybean 305423 oil.

As expected, the intake of oleic acid, and consequently that of MUFA, considerably increased in all age groups when commercial vegetable oils were replaced by soybean 305423 oil. No dietary reference value has been set for MUFA by EFSA (see EFSA NDA Panel, 2010). However, the calculated increased intakes of MUFA (16 E % vs. 12 E %) are in the range of those observed for adults in EU countries (11–21 E %).

Linoleic acid (LA) is the main dietary n-6 PUFA in the human diet. EFSA has proposed an adequate intake (AI) for LA of 4 E %, based on the lowest estimated mean intakes of the various population groups from a number of European countries, where LA deficiency symptoms are not present. This AI corresponds to 9 g linoleic acid/day for an energy intake of 2 000 kcal. Replacement of vegetable oil with soybean 305423 oil would result in 4 E % for adults and the elderly. Reduction below the AI was observed for toddlers, children and teenagers (3.2–3.8 E %). The EFSA GMO Panel is of the opinion that this is not a matter of concern, as LA deficiency symptoms have not been observed at intakes > 1 E % (EFSA NDA Panel, 2010). As no baseline data for the LA intake at the percentile below the mean were available, an estimate on the consequences of the replacement of vegetable oils with the soybean 305423 oil for the population sub-groups could not be made.

Alpha-linolenic acid (ALA) is the main dietary n-3 PUFA. EFSA has proposed an AI for ALA of 0.5 E %, based on the lowest estimated mean intakes of the various population groups from a number of European countries, where ALA deficiency symptoms are not present. The intake of n-3 PUFA, predominantly ALA, was found to be between 1.9–2.3 g/day in adult men (0.7–1.2 E %) and 1.5–1.8 g/day in women (0.7–1.2 E %) in five European countries (EFSA NDA Panel, 2010). Replacement of vegetable oils with soybean 305423 oil was calculated to result in an increase of the n-3 PUFA consumption for all age groups (see Tables 1 and 2). The Panel notes that the 9,15 isomer of LA contributes to the n-3 PUFA values taken for the exposure scenario. However, this isomer cannot be converted to α-linolenic acid, because humans lack Δ12 desaturase. As the 9,15 ALA isomer amounts to less than 10 % of the total n-3 PUFA, it has little impact on the main outcome.

TFA is considered undesirable and, consequently, the intake should not exceed 1 E %, as is recommended by international and national authorities. The mean TFA consumption of all age groups at baseline was below this value and did not appreciably change after replacement of vegetable oils with soybean 305423 oil (see Tables 1 and 2). This is also true for high consumers (97.5th percentile).[45]

5.1.5.2. Odd chain fatty acids

The dominant odd chain fatty acids (heptadecanoic (C17:0), heptadecenoic (C17:1) and isomer 1 and 2 of nonadecenoic acid (C19:1)) sum to 2.7 % in the soybean 305423, compared with about 0.3 % in the conventional counterpart. On request of the EFSA GMO Panel, the applicant provided an exposure assessment for the odd chain fatty acids. A daily soybean oil consumption *per capita* of 36 g/day was calculated as the mean of 23 European Union (EU) countries based on the FAOSTAT databases as well as annual production and trade data (1961–2005) (FAOSTAT, 2005). However, the basis for this assessment was analytical values of the refined–bleached–deodorised soybean 305423 oil taken from the 2005 field trial. Recalculating using the 2011 data on which the exposure assessment (section 5.1.4.1) is based indicated the average daily intake of 0.9 g of odd chain fatty acids from soybean 305423 compared with 0.1 g from its conventional counterpart.

[45] Study report 2013.

Odd chain fatty acids are normal constituents of the human diet (soy products, shortening, margarine, tofu, butter, pork, beef and lamb).[46] There appear to be no published studies on the catabolism of these odd chain fatty acids in mammals. However, it is thought to be likely that they are metabolised by β-oxidation, like even chain fatty acids. The terminal metabolite is expected to be propionyl-CoA (instead of acetyl-CoA). Heptadecanoic acid and heptadecenoic acid are also found in human tissues, (Shenolikar, 1980; Wendel, 1989; Andersson et al., 2002; Baylin et al., 2002).

5.1.5.3. Conclusion on nutritional impact in humans

Full replacement of vegetable oils with oil derived from soybean 305423 would not change substantially the average intake of SFA and n-3 PUFA, but would increase MUFA and odd chain fatty acids, and decrease n-6 PUFA intake. These changes are small and without impact on health and nutrition.

Other soybean products for human consumption are not expected to differ in their composition, except for their fatty acids content. The contribution of fatty acids from such products to overall human exposure would be small and is not expected to affect the conclusion on human health and nutrition.

5.1.5.4. Animal nutritional assessment[47]

Presently, only small amounts of full-fat soybeans (1 % of the total soybean feed) are directly fed to food-producing animals. The use of soybean oil in animal feed is limited and only small amounts (0.5–3 %) are added to mixed feed (especially for poultry and pigs) in order to avoid dust, improve the quality/stability of pellets and add energy to the diets. Defatted toasted soybean meal represents the most common soybean byproduct used in zootechnical animal feed formulations, with around 90 % of the defatted soybean meal entering the feed chain in the EU, mainly to poultry, pigs and cattle.[48]

In the animal feeding studies provided by the applicant, diets included mainly toasted defatted soybean meals from soybean 305423, the conventional counterpart (in the laying hen and pig studies), the negative segregant (in the rat and chicken studies) and non-GM commercial varieties. Soybean 305423 oil (with the expected compositional changes in fatty acids profile) was tested at a low inclusion rate (0.5 %) in the chicken study only. Diets were designed to deliver the same nutrition as the concurrent control diets and, overall, no significant differences were noted between groups. Furthermore, in the food-producing species tested (chickens, laying hens and pigs) performance parameters fell within the ranges defined by data from concurrent groups fed diets containing commercial non-GM soybean meal varieties (see section 5.1.3). The EFSA GMO Panel is of the opinion that the incorporation of feeding stuff derived from soybean 305423 to nutritionally balanced diets has no impact on health and performance of the tested species.

5.1.6. Post-market monitoring of GM food/feed

A proposal for a post-market monitoring (PMM) plan needs to be provided by the applicant (EFSA, 2006b, 2011c). EFSA recommends that the PMM should focus on the collection of consumption data for the European population.

5.2. Conclusion

Full replacement of vegetable oils with oil derived from soybean 305423 would not change substantially the average intake of SFA and n-3 PUFA, but would increase the average intake of MUFA and odd chain fatty acids, and decrease n-6 PUFA intake. These changes in average intake are small and without impact on health and nutrition. The contribution of fatty acids from soybean 305423 in other soybean products to overall human exposure would be small and is not expected to affect the conclusion on human health and nutrition.

[46] Additional information, April 2008.
[47] Technical Dossier/Sections D7.8.3 and D7.10.2.
[48] Personal communication from Deutscher Verband für Tiernahrung, 29 July 2011.

The safety assessment identified no concerns regarding the potential toxicity and allergenicity of the newly introduced GM-HRA protein. There are no indications that the genetic modification might significantly change the overall allergenicity of soybean 305423 when compared with that of its conventional counterpart.

Based on the results of studies in chickens for fattening, laying hens, pigs and rats, it is concluded that feeding stuffs derived from soybean 305423 are safe and as nutritious as those derived from other non-GM soybean varieties for all animal species.

6. Environmental risk assessment and monitoring plan

6.1. Evaluation of relevant scientific data

Considering the scope of application EFSA-GMO-NL-2007-45, the environmental risk assessment (ERA) of soybean 305423 is concerned mainly with ingestion by animals and their manure and faeces leading to exposure of the gastrointestinal tract and soil microorganisms to recombinant DNA and with accidental release into the environment of viable soybean 305423 grains during transport and processing.

As the scope of the present application excludes cultivation, environmental concerns in the EU related to the use of ALS-inhibiting herbicides on soybean 305423 do not apply.

6.1.1. Unintended effects on plant fitness due to the genetic modification[49]

Cultivated soybean (*Glycine max* (L.) Merr.) is a species in the sub-genus *Soja* of the genus *Glycine*. The species originated from eastern Asia and is a highly domesticated crop (Lu, 2005). The major worldwide soybean producers are Argentina, Brazil, China, North Korea, South Korea and the USA. In the EU,[50,51] soybean is mainly cultivated in Italy, Romania, France, Hungary, Austria, Slovakia and the Czech Republic (Dorokhov et al., 2004; Krumphuber, 2008). Cultivated soybean seeds rarely display any dormancy characteristics, and only under certain environmental conditions grow as volunteers in the year following cultivation. If volunteers occur, they do not compete well with the succeeding crop, and can easily be controlled mechanically or chemically (OECD, 2000). In soybean fields, seeds usually do not survive during the winter owing to predation, rotting and germination resulting in death, or owing to management practices prior to planting the subsequent crop (Owen, 2005).

For the assessment of potential impacts on the environment in case of accidental release of GM soybean grains during transport and processing, the EFSA GMO Panel mainly considered the comprehensive 2011 dataset in the USA and Canada (for further details, see section 4.1.2). Laboratory tests and field studies at several locations in the USA (nine locations) and Canada (one location) in 2011 have been carried out to assess the phenotypic and agronomic characteristics as well as ecological interactions of GM soybean 305423 in comparison with an appropriate comparator and several non-GM soybean reference varieties. Considering the scope of the application, special attention is paid to those agronomic characteristics (e.g. early and final plant population, seedling vigour, and yield) which may affect the survival, establishment and fitness of the GM soybean grains which could be accidentally released into the environment.

Some statistically significant differences (e.g. for early and final plant population, plant height, shattering, seedling vigour and yield) were observed in the across-location statistical analysis of the 2011 dataset in the USA and Canada. Both the soybean 305423 samples sprayed with the intended herbicide and the samples sprayed with conventional herbicides had lower early and final stand counts, seedling vigour and yield than their comparators. The equivalence test indicated that all the analysed characteristics fell within the equivalence limits established from the non-GM soybean

[49] Technical dossier/Section D9.1.
[50] Available online: http://faostat.fao.org/site/567/default.aspx#ancor
[51] Available online: http://epp.eurostat.ec.europa.eu/portal/page/portal/agriculture/data/database

reference varieties, except for lodging when soybean 305423 was sprayed with conventional herbicides (see section 4.1.3). The observed differences are not considered environmentally relevant in the context of the scope of this application, as they suggest a lower fitness of soybean 305423. The EFSA GMO Panel considers that the differences observed are unlikely to affect the overall fitness, invasiveness or weediness of the GM soybean, except under conditions of application of the intended herbicide.

The herbicide tolerance trait can be regarded as providing only a potential agronomic and selective advantage to this GM soybean plant where and when ALS-inhibiting herbicides are applied. However, survival of soybean plants outside cultivation where ALS-inhibiting herbicides are applied is mainly limited by a combination of low competitiveness, absence of a dormancy phase and susceptibility to plant pathogens and cold climatic conditions. As these general characteristics are unchanged in soybean 305423, herbicide tolerance is not likely to provide a selective advantage outside cultivation. Even if ALS-inhibiting herbicides are applied to these plants, it will not change their ability to survive over seasons. Therefore, it is considered very unlikely that soybean 305423 will differ from conventional soybean varieties in its ability to survive until subsequent seasons or to establish feral populations under European environmental conditions.

Therefore, from the data presented in the application, there is no indication of an increased persistence and invasiveness potential of soybean 305423 compared with conventional soybean and it can be considered that soybean 305423 has no altered survival, multiplication or dissemination characteristics compared with its conventional counterpart, except under application of ALS-inhibiting herbicides.

In addition to the data presented by the applicant, the EFSA GMO Panel is not aware of any scientific report of increased spread and establishment of existing GM soybeans and any change in survival capacity, including overwintering (Dorokhov et al., 2004; Owen, 2005; Bagavathiannan and Van Acker, 2008; Lee et al., 2008).

Therefore, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects of soybean 305423 in Europe will not be different to that of conventional soybean varieties.

6.1.2. Potential for gene transfer[52]

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 vertical gene flow via seed dispersal and cross-pollination.

6.1.2.1. Plant-to-bacteria gene transfer

Genomic DNA is a component of many 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 animals feeding on the GM soybean is expected.

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

A successful horizontal transfer would require stable insertion of the transgene sequences into a bacterial genome and a selective advantage conferred to the transformed host. The only known mechanism that facilitates horizontal transfer of non-mobile, chromosomal DNA fragments into bacterial genomes is homologous recombination. This requires the presence of stretches of DNA sequences that are similar in the recombining DNA molecules and, in addition to substitutive gene

[52] Technical dossier/Section D9.2.

replacement, facilitates the insertion of non-homologous DNA sequences if their flanking regions share sequence similarity with bacterial sequences in the recipient.

Soybean 305423 contains a *gm-fad2-1* gene fragment leading to a decreased level of the fatty acid desaturase and the *gm-hra* gene which confers tolerance to ALS-inhibiting herbicides. Both *gm-fad2-1* and *gm-hra* genes, as well as their regulatory sequences, are of soybean origin. Furthermore, soybean 305423 was obtained by particle bombardment. The analyses of the recombinant DNA revealed the presence of a 495-bp-long sequence originating from the plasmid PHP19340 or PHP17752, which must be of bacterial origin. This fragment, however, is considered to be a non-functional fragment; thus, it is not a regulatory element and it does not encode a functional protein. Therefore, in the very unlikely but theoretically possible case of transfer of this gene facilitated by homologous recombination or also by illegitimate recombination to natural plasmids, no trait would be conferred.

Owing to the plant origin of all genetic elements of the inserts encoding for functional genes in soybean 305423, no increased likelihood for homologous recombination compared to DNA from non-GM soybean were identified. Furthermore, no risk was identified for the unlikely but theoretically possible transfer of the non-functional DNA fragment of bacterial origin to plasmids as they may occur in environmental bacteria.

6.1.2.2. Plant-to-plant gene transfer

Considering the scope of this application and physical characteristics of soybean seeds, a possible pathway of gene dispersal is from grain spillage and pollen of occasional feral GM soybean plants originating from accidental grain spillage during transport and/or processing.

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 in 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* (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). However, as *G. soja* and *G. gracilis* are indigenous to Australia, China, Japan, Korea, the Philippines, the far eastern region of Russia, the South Pacific and Taiwan, and as 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 in the EU is restricted to cultivated soybean.

Soybean (*Glycine max*) is an annual, almost completely self-pollinating crop in the field, with a percentage of cross-pollination usually lower than 1 % (Weber and Hanson, 1961; Caviness, 1966; 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. 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 abundance of pollinators (Gumisiriza and Rubaihayo, 1978; Kikuchi et al., 1993; Ahrent and Caviness, 1994; Ray et al., 2003; Lu, 2005).

Plant-to-plant gene transfer could therefore occur under the following scenarios: imports of viable soybean 305423 grains (while most soybean 305423 grains will be processed in countries of production), processing outside of importing ports, transportation in regions of soybean production in Europe, spillage of GM grains during transport, germination and development of spilled grains within soybean fields or in very close vicinity of cultivated soybean fields, overlap of flowering periods and environmental conditions favouring cross-pollination. The overall likelihood of cross-pollination

between GM soybean plants and cultivated soybean is therefore extremely low. Apart from seed production areas, GM plants and plants derived from out-crossing with this GM soybean will not persist overtime. Dispersal of soybean seeds by animals is not expected owing to the characteristics of the seed, but accidental release into the environment of grains may occur during transport and processing for food, feed and industrial uses. However, cultivated soybean seeds rarely display any dormancy characteristics and only under certain environmental conditions grow as volunteers in the year following cultivation. If volunteers occur, they do not compete well with the succeeding crop, and can easily be controlled mechanically or chemically (OECD, 2000). Even 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).

The EFSA GMO Panel takes into account the fact that this application does not include cultivation of the soybean within the EU so that the likelihood of cross-pollination between cultivated soybean and occasional soybean plants resulting from seed spillage is considered extremely low. However, in countries cultivating this GM soybean and producing seed for export, there is a potential for admixture in seed production and thus the introduction of GM seeds through this route.

In conclusion, as there are no significant changes of overall fitness, invasiveness or weediness of soybean 305423 with respect to conventional soybean, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes from soybean 305423 in Europe will not differ from that of conventional soybean varieties.

6.1.3. Interactions of the GM plant with target organisms[53]

Considering the scope of this application, excluding cultivation, and the absence of target organisms, potential interactions of the GM plant with target organisms were not considered an issue by the EFSA GMO Panel.

6.1.4. Interactions of the GM plant with non-target organisms[54]

Considering the scope of this application, excluding cultivation, and the low level of exposure to the environment, potential interactions of soybean 305423 with non-target organisms were not considered an issue by the EFSA GMO Panel.

6.1.5. Interactions with the abiotic environment and biogeochemical cycles[55]

Considering the scope of this application, excluding cultivation, and the low level of exposure to the environment, potential interactions of the GM plant with the abiotic environment and biogeochemical cycles were not considered an issue by the EFSA GMO Panel.

6.2. Post-market environmental monitoring[56]

The objectives of a post-market environmental monitoring (PMEM) plan according to Annex VII of Directive 2001/18/EC are (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the 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 ERA.

Monitoring is related to risk management and thus a final adoption of the PMEM plan falls outside the mandate of EFSA. However, the EFSA GMO Panel gives its opinion on the scientific content of the monitoring plan provided by the applicant (EFSA, 2011). The potential exposure to the environment of soybean 305423 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

[53] Technical dossier/sections D8 and D9.4.
[54] Technical dossier/section D9.5.
[55] Technical dossier/sections D9.8 and D10.
[56] Technical dossier/section D11.

accidental release into the environment of GM soybean grains during transport and/or processing. The PMEM plan provided by the applicant is in line with the scope of the application. As the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects, no case-specific monitoring is necessary.

The PMEM 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 GM organisms 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 use of networks of existing surveillance systems. The applicant proposes to submit a PMEM report on an annual basis and a final report at the end of the consent.

The EFSA GMO Panel is of the opinion that the PMEM plan proposed by the applicant is in line with the scope of the application as the ERA of soybean 305423 did not cover cultivation and identified no potential adverse environmental effects (EFSA, 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 case of accidental release of viable grains of soybean 305423. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the PMEM plan.

6.3. Conclusion

Considering the scope of application EFSA-GMO-NL-2007-45, the ERA of soybean 305423 is concerned with indirect exposure, mainly through ingestion by animals and their manure and faeces leading to exposure of the gastrointestinal tract and soil bacteria to recombinant DNA and through the accidental release into the environment of viable soybean 305423 grains (e.g. during transport and/or processing).

In the case of accidental release into the environment of viable grains of soybean 305423, there are no indications of an increased likelihood of establishment and spread of feral soybean 305423 plants, except in the presence of ALS-inhibiting herbicides. Considering the scope of the application, potential interactions of soybean 305423 with the biotic and abiotic environment were not considered to be an issue by the EFSA GMO Panel. Furthermore, the EFSA GMO Panel is of the opinion that the unlikely but theoretically possible transfer of the recombinant gene from soybean 305423 to environmental bacteria does not raise concern owing to the lack of a selective advantage in the context of the scope of the application.

The PMEM plan provided by the applicant is in line with the scope of the application and the Guidance document of the EFSA GMO Panel on PMEM of GM plants (EFSA, 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 case of accidental release of viable grains of soybean 305423. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The EFSA GMO Panel was requested to carry out a scientific assessment of soybean 305423 for food and feed uses, import and processing in accordance with Regulation (EC) No 1829/2003.

The EFSA GMO Panel is of the opinion that the molecular characterisation data provided for soybean 305423 are sufficient to conclude on this part of the risk assessment evaluation. The results of the bioinformatic analyses of the inserted DNA and the flanking regions do not raise safety issues. The levels of *Glycine max* herbicide-resistant ALS (GM-HRA) protein in soybean 305423 have been sufficiently analysed in various tissues. One plant out of the 1 100 tested individuals had undergone recombination and, as a result, had lost the entire *gm-hra* cassette and portions of the promoter

elements flanking the cassette. However, the EFSA GMO Panel is of the opinion that this recombination, leading to loss of the trait, raises no safety issues. The stability of the inserted DNA was confirmed over several generations. The EFSA GMO Panel considers that the molecular characterisation does not indicate a safety issue.

The EFSA GMO Panel compared the compositional, agronomic and phenotypic characteristics of soybean 305423, with its conventional counterpart and non-GM reference soybean varieties and assessed all statistically significant differences between soybean 305423 and its conventional counterpart, for which equivalence with the non-GM reference varieties could not be established. The EFSA GMO Panel concludes that the composition of soybean 305423 differs from that of the conventional counterpart and that of non-GM reference varieties in its fatty acid profile, the newly expressed protein *max* herbicide-resistant ALS (GM-HRA), consistently with the objective of the modification as well as with the expression of the ALS enzyme of soybean 305423; differences in the minerals zinc and calcium and the isoflavone glycitin were also noted, and for these no further assessment was deemed necessary owing to their well-known biochemical roles and to the magnitude of the reported levels. The EFSA GMO Panel also concludes that no differences were identified in the agronomic and phenotypic characteristics that would require further assessment with regard to safety.

Full replacement of vegetable oils with oil derived from soybean 305423 would not change substantially the average intake of SFA and n-3 PUFA, but would increase the average intake of MUFA and odd chain fatty acids, and decrease n-6 PUFA intake. These changes in the average intake are small and without impact on health and nutrition. The contribution of fatty acids from soybean 305423 in other soybean products to overall human exposure would be small and is not expected to affect the conclusion on human health and nutrition.

The safety assessment identified no concerns regarding the potential toxicity and allergenicity of the newly introduced GM-HRA protein. There are no indications that the genetic modification might significantly change the overall allergenicity of soybean 305423 when compared with that of its conventional counterpart.

Based on the results of studies in chickens for fattening, laying hens, pigs and rats, it is concluded that feeding stuffs derived from soybean 305423 are safe and as nutritious as those derived from other non-GM soybean varieties for all animal species.

Considering the intended altered soybean 305423 nutritional composition, a proposal for a post-market monitoring (PMM) plan needs to be provided by the applicant (EFSA, 2006b, 2011c). EFSA recommends that the PMM should focus on the collection of consumption data for the European population.

The scope of application EFSA-GMO-NL-2007-45 is for food and feed uses, import and processing and does not include cultivation. Therefore, there is no requirement for scientific information on possible environmental effects associated with the cultivation of soybean 305423 in Europe. There are no indications of an increased likelihood of establishment and spread of feral GM soybean plants in the case of accidental release into the environment of viable soybean 305423 grains during transport and processing for food and feed uses, except in the presence of ALS-inhibiting herbicides. Considering the scope of this application, potential biotic and abiotic interactions of soybean 305423 were not considered to be an issue by the EFSA GMO Panel. The unlikely but theoretically possible transfer of the recombinant gene from soybean 305423 to environmental bacteria does not raise any safety concerns because no selective advantage will be conferred to the recipients. The scope of the post-market environmental monitoring plan provided by the applicant is in line with the scope of the application. Furthermore, the EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the post-market environmental monitoring plan.

In conclusion, the EFSA GMO Panel considers that the information available for soybean 305423 addresses the scientific issues indicated by the Guidance document of the EFSA GMO Panel and the

scientific comments raised by the Member States, and that soybean 305423 is as safe as its conventional counterpart and is unlikely to have adverse effects on human and animal health and the environment in the context of the scope of this application.

Considering the altered composition and nutritional values of soybean 305423, the EFSA GMO Panel considered a specific labelling proposal provided by the applicant in accordance with Articles 13(2)(a) and 25(2)(c) of Regulation (EC) No 1829/2003. The applicant proposed that food and feed products within the scope of the application should be labelled as "genetically modified soybean with altered fatty acid profile". The GMO Panel is of the opinion that the compositional data (see section 4.1.3 above) show that the fatty acid composition of seeds of soybean 305423 and derived oil has indeed been changed in relation to the conventional counterpart.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of the Netherlands, received 18 June 2007, concerning a request for placing on the market of soybean 305423 in accordance with Regulation (EC) No 1829/2003.

2. Acknowledgement letter, dated 22 June 2007, from EFSA to the Competent Authority of the Netherlands.

3. Letter from EFSA to applicant, dated 28 September 2007, requesting additional information under completeness check.

4. Letter from applicant to EFSA, received 12 October 2007, providing additional information under completeness check.

5. Letter from EFSA to applicant, dated 22 October 2007, delivering the "Statement of Validity" for application EFSA-GMO-NL-2007-45, soybean 305423 submitted by Pioneer under Regulation (EC) No 1829/2003.

6. Letter from EFSA to applicant, dated 20 December 2007, requesting additional information and stopping the clock.

7. Letter from applicant to EFSA, received 12 February 2007, providing additional information.

8. Letter from EFSA to applicant, dated 28 February 2008, requesting additional information and maintaining the clock stopped.

9. Letter from applicant to EFSA, received 28 April 2008, providing additional information.

10. Letter from EFSA to applicant, dated 22 May 2008, requesting additional information and maintaining the clock stopped.

11. Letter from applicant to EFSA, received 4 July 2008, providing additional information.

12. Letter from EFSA to applicant, dated 13 August 2008, requesting additional information and maintaining the clock stopped.

13. Letter from applicant to EFSA, received 24 November 2008, providing additional information.

14. Letter from EFSA to applicant, dated 11 February 2009, requesting additional information and maintaining the clock stopped.

15. Letter from applicant to EFSA, received 19 March 2009, providing additional information.

16. Letter from applicant to EFSA, received 6 October 2009, providing additional information.

17. Letter from EFSA to applicant, dated 8 January 2010, requesting additional information and maintaining the clock stopped.

18. Letter from applicant to EFSA, received 9 February 2010, providing additional information.

19. Letter from EFSA to applicant, dated 27 April 2010, requesting additional information and maintaining the clock stopped.

20. Letter from applicant to EFSA, received 6 June 2010, providing additional information.

21. Letter from EFSA to applicant, dated 5 August 2010, requesting additional information and maintaining the clock stopped.

22. Letter from applicant to EFSA, received 21 September 2010, providing additional information.
23. Letter from EFSA to applicant, dated 21 October 2010, requesting additional information and maintaining the clock stopped.
24. Letter from applicant to EFSA, received 18 November 2010, requesting clarifications on the additional information requested by EFSA.
25. Letter from EFSA to applicant, dated 22 December 2010, providing clarifications.
26. Letter from applicant to EFSA, received 9 February 2011, providing the additional information requested.
27. Letter from EFSA to applicant, dated 17 May 2011, re-starting the clock.
28. Letter from applicant to EFSA, received 14 July 2011, providing clarifications requested by the EC.
29. Letter from applicant to EFSA, received 28 November 2011, requesting clarifications to EFSA.
30. Letter from applicant to EFSA, received 3 January 2012, requesting clarifications to EFSA.
31. Letter from EFSA to applicant, dated 12 January 2012, providing clarifications.
32. Letter from applicant to EFSA, received 17 January 2012, providing additional information.
33. Letter from applicant to EFSA, received 5 March 2012, providing additional information.
34. Letter from EFSA to applicant, dated 30 May 2012, requesting additional information and maintaining the clock stopped.
35. Letter from applicant to EFSA, received 5 June 2012, providing additional information.
36. Letter from EFSA to applicant, dated 19 September 2012, requesting additional information and maintaining the clock stopped.
37. Letter from applicant to EFSA, received 31 October 2012, providing additional information.
38. Letter from EFSA to applicant, dated 8 February 2013, requesting additional information and maintaining the clock stopped.
39. Letter from EFSA to applicant, dated 20 February 2013, requesting additional information and maintaining the clock stopped.
40. Letter from EFSA to applicant, dated 12 April 2013, requesting additional information and maintaining the clock stopped.
41. Letters from applicant to EFSA, received 2 May 2013, providing additional information.
42. Letter from EFSA to applicant, dated 26 June 2013, requesting additional information and maintaining the clock stopped.
43. Letter from applicant to EFSA, received 28 June 2013, providing the additional information requested.
44. Letter from EFSA to applicant, dated 5 September 2013, re-starting the clock.
45. Letter from applicant to EFSA, received 3 October 2013, providing additional information.

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, Junior JN, Monteiro P, Rech EL and Aragao 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.

Al-Tawaha AM, Seguin P, Smith DL and Bonnell RB, 2007. Irrigation level affects isoflavone concentrations of early maturing soya bean cultivars. Journal of Agronomy and Crop Science, 193, 238–246.

Andersson A, Nälsén C, Tegblad S and Vessby B, 2002. Fatty acid composition of skeletal muscle reflects dietary composition in humans. American Journal of Clinical Nutrition, 76, 1222–1229.

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

Baylin A, Kabagambe EK, Siles X and Campos H, 2002. Adipose tissue biomarkers offatty acid intake. American Journal of Clinical Nutrition, 76, 750–757.

Bates B, Lennox A, Bates C and Swan G, 2011. National diet and nutrition survey, Headline results from year 1 and year 2 (combined) of the rolling program (2008/2009–2009/2010). DH-FSA. Available online: https://www.gov.uk/government/publications/national-diet-and-nutrition-survey-headline-results-from-years-1-and-2-combined-of-the-rolling-programme-2008-9-2009-10

Caviness CE, 1966. Estimates of natural cross-pollination in Jackson soybeans in Arkansas. Crop Science, 6, 211–212.

Codex Alimentarius, 2009. Foods derived from modern biotechnology. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Rome, Italy, 85 pp.

Delaney B, Appenzellar L, Munley S, Hoban D, Sykes G, Malley L and Sanders C, 2008. Subchronic feeding study of high oleic acid soybeans (Event DP-305423–1) in Sprague–Dawley rats. Food and Chemical Toxicology, 46, 3808–3817.

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: Introgression from genetically modified plants into wild relatives. Eds Den Nijs H, Bartsch D and Sweet J. CABI Publishing, Wallingford, UK, 151–161.

EFSA (European Food Safety Authority), 2006a. Guidance document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed. The EFSA Journal 2006, 99, 1–100.

EFSA (European Food Safety Authority), 2006b. Opinion of the Scientific Panel on Genetically Modified Organisms on the Post Market Environmental Monitoring (PMEM) on Genetically Modified Plants. The EFSA Journal 2006, 319, 1–27.

EFSA (European Food Safety Authority), 2009. Consolidated presentation of the joint Scientific Opinion of the GMO and BIOHAZ Panels on the "Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants" and the Scientific Opinion of the GMO Panel on "Consequences of the Opinion on the Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants on Previous EFSA Assessments of Individual GM Plants". The EFSA Journal 2009, 1108, 1–8.

EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA), 2010. Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids,

monounsaturated fatty acids, trans fatty acids, and cholesterol. EFSA Journal 2010;8(3):1461, 107 pp. doi:10.2903/j.efsa.2010.1461

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

EFSA Panel on Genetically Modified Organisms (GMO), 2010b. Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal 2010;8(7):1700, 168 pp. doi:10.2903/j.efsa.2010.1700

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

EFSA Panel on Genetically Modified Organisms (GMO), 2011b. Opinion of the Scientific Panel on Genetically Modified Organisms on application (EFSA-GMO-UK-2007-43) for the placing on the market of herbicide tolerant genetically modified soybean 356043 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Pioneer. EFSA Journal 2011;9(7):2310, 40 pp. doi:10.2903/j.efsa.2011.2310

EFSA Panel on Genetically Modified Organisms (GMO), 2011c. Scientific Opinion on guidance on the Post-Market Environmental Monitoring (PMEM) of genetically modified plants. EFSA Journal 2011;9(8):2316, 43 pp. doi:10.2903/j.efsa.2011.2316

FAOSTAT (Food and Agricultural Organization of the United Nations—Statistics Division), 2005. Production Quantities of Soybean.

Gumisiriza G and Rubaihayo PR, 1978. Factor that influence outcrossing in soybean. Zeitschrift fuer Acker und Pflanzenbau—Journal of Agronomy and Crop Science, 147, 129–133.

Gutierrez-Gonzalez J, Wu X, Zhang J, Lee J-D, Ellersieck M, Shannon J, Yu O, Nguyen H and Sleper D, 2009. Genetic control of soybean seed isoflavone content: importance of statistical model and epistasis in complex traits. Theoretical and Applied Genetics, 119, 1069–1083.

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.

Krumphuber C, 2008. Cultivating soybean in Austria and Europe—a situation analysis. In: 1. Austrian Soy Symposium (135 Years of Soybean and Soy Research), 9–10.

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 für Verbraucherschutz und Lebensmittelsicherheit—Journal of Consumer Protection and Food Safety, 2, 25–28.

Lee B, Kim C, Park J, Park K, Kim H, Yi H, Jeong S, Yoon W and Kim H, 2008. Monitoring the occurrence of genetically modified soybean and maize in cultivated fields and 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: Crop Ferality and Volunteerism. Ed. Gressel J**.** 137–147.

McNaughton J, Roberts M, Smith B, Rice D, Hinds M, Sanders C, Layton R, Lamb I and Delaney B, 2008. Comparison of broiler performance when fed diets containing event DP-3O5423–1, nontransgenic near-isoline control, or commercial reference soybean meal, hulls, and oil. Poultry Science, 87, 2549–2561.

Mejia L, Jacobs CM, Utterback PL, Parsons CM, Rice D, Sanders C, Smith B, Iiams C and Sauber T, 2010. Evaluation of the nutritional equivalency of soybean meal with the genetically modified trait DP-3Ø5423–1 when fed to laying hens. Poultry Science, 89, 2634–2639.

Miller PS, Rice DW, Smith BL, Sanders C, Byndy JW and Moreno R. 2011. Evaluation of soybean meal with the genetically modified trait DP-3Ø5423-1 fed to growing pigs. Nebraska Pig Report, 24–27.

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.

NRC (National Research Council), 1994. Nutrients requirements of poultry. 9th rev. edn. National Academies Press, Washington, DC, USA.

NRC (National Research Council), 1998. Nutrients requirements of swine. 10th rev. edn. National Academies Press, Washington, DC, USA.

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, No 15. ENV/JM/MONO(2000)9.

OECD (Organisation for Economic Co-operation and Development), 2001. Consensus document on compositional considerations for new varieties of soybean: key food and feed nutrients and anti-nutrients. ENV/JM/MONO(2001)15.

Owen MDK, 2005. Maize and soybeans—controllable volunteerism without ferality? In: Crop ferality and volunteerism. Ed. Gressel J. 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.

Shenolikar I, 1980. Fatty acid profile of myocardial lipid in populations consuming different dietary fats. Lipids, 15, 980–982.

Weber CR and Hanson WD, 1961. Natural hybridization with and without ionizing radiation in soybeans. Crop Science, 1, 389–392.

Wendel U, 1989. Abnormality of odd-numbered long-chain fatty acids in erythrocyte membrane lipids from patients with disorders of propionate metabolism. Pediatric Research, 25, 147–150.

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 für Verbraucherschutz und Lebensmittelsicherheit—Journal of Consumer Protection and Food Safety, 3, 14–16.

Yoshimura Y, Beckie HJ and Matsuo K, 2006. Transgenic oilseed rape along transportation routes and port of Vancouver in western Canada. Environmental Biosafety Research, 5, 67–75.