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Scientific Opinion on an application by Pioneer (EFSA-GMO-NL-2007-47) for the placing on the market of the herbicide-tolerant, high-oleic acid, genetically modified soybean 305423 × 40-3-2 for food and feed uses, import and processing under Regulation (EC) No 1829/2003

EFSA Panel on Genetically Modified Organisms (GMO)

Abstract

The Panel on Genetically Modified Organisms of the European Food Safety Authority (GMO Panel) previously assessed the two single events combined to produce soybean 305423 × 40-3-2 and did not identify safety concerns. No new data on the single events affecting the original conclusions were identified. Based on the molecular, agronomic, phenotypic and compositional characteristics, the combination of soybean events 305423 and 40-3-2 in the two-event stack soybean did not raise concerns regarding food and feed safety or nutrition. The combination of the newly expressed proteins in the two-event stack soybean did not raise human or animal health concerns. No compositional differences requiring further assessment were identified between soybean 305423 × 40-3-2, the non-GM comparator, additional comparators and the non-GM commercial soybean reference varieties, except for the altered fatty acid profile (consistent with the intended trait). Nutritional assessment of food products from soybean 305423 × 40-3-2 identified no concerns for human health and nutrition. There are no concerns regarding the use of feedingstuffs from defatted toasted soybean 305423 × 40-3-2 meal. There are no indications of an increased likelihood of establishment and spread of occasional feral soybean plants, unless these are exposed to acetolactate-synthase-inhibiting or glyphosate-containing herbicides. Risks associated with the unlikely, but theoretically possible, horizontal transfer of recombinant genes from soybean 305423 × 40-3-2 to bacteria were not identified. Considering the scope of the application, interactions with biotic and abiotic environments are not considered a relevant issue. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of soybean 305423 × 40-3-2. The GMO Panel is of the opinion that soybean 305423 × 40-3-2 is as safe as the non-GM comparator and non-GM commercial soybean varieties with respect to potential effects on human and animal health and environment in the context of its scope. The GMO panel recommends a post-market monitoring plan.

Keywords: GMO, soybean 305423 × 40-3-2, herbicide tolerant, high oleic acid, GM-HRA, CP4 EPSPS, RNAi, Regulation (EC) No 1829/2003

Requestor: Competent Authority of the Netherlands

Question number: EFSA-Q-2007-175

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Summary

Following the submission of application EFSA-GMO-NL-2007-47 under Regulation (EC) No 1829/2003 from Pioneer, the Panel on Genetically Modified Organisms of the European Food Safety Authority (GMO Panel) was asked to deliver a scientific opinion on the safety of the herbicide-tolerant, high-oleic acid, genetically modified (GM) soybean 305423 × 40-3-2 (Unique Identifier DP-3Ø5423-1xMON-Ø4Ø32-6). The scope of application EFSA-GMO-NL-2007-47 is for food and feed uses, import and processing, but excludes cultivation within the European Union (EU).

The single soybean events 305423 (conferring the high-oleic acid phenotype and expressing *Glycine max* herbicide-resistant ALS (GM-HRA)) and 40-3-2 (expressing CP4 EPSPS) were assessed previously by EFSA and no concerns were identified for human and animal health or environmental safety. No safety issue was identified by updated bioinformatic analyses, nor reported by the applicant concerning the two single soybean events, since the publication of the respective scientific opinions. Consequently, the GMO Panel considers that the previous conclusions on the safety of the single soybean events remain valid.

The two-event stack soybean 305423 × 40-3-2 was produced by conventional crossing to produce high-oleic acid phenotype soybean tolerant to acetolactate-synthase (ALS)-inhibiting herbicides and glyphosate-based herbicides. The GMO Panel evaluated soybean 305423 × 40-3-2 with reference to the scope of the application and the appropriate principles described in its guidelines for the risk assessment of GM plants and derived food and feed, the environmental risk assessment (ERA) of GM plants and the post-market environmental monitoring (PMEM) of GM plants. The scientific evaluation of the risk assessment included molecular characterisation of the inserted DNA and analysis of the expression of the corresponding proteins. An evaluation of the comparative analyses of the compositional, agronomic and phenotypic characteristics was undertaken, and the safety of the newly expressed proteins and of the whole food/feed was evaluated with respect to potential toxicity, allergenicity and nutritional wholesomeness. An evaluation of environmental impacts and the PMEM plan was also undertaken. In accordance with the GMO Panel guidance documents applicable to this application (EFSA, 2007), 'Where all single events have been assessed, the risk assessment of stacked events should focus mainly on issues related to (a) stability, (b) expression of the events and (c) potential interactions between the events'.

The molecular data establish that the events stacked in soybean 305423 × 40-3-2 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins in the two-event stack and the parental lines are similar or present differences that are not unexpected. There is no indication of an epistatic interaction between the events that may affect the levels of the newly expressed proteins. No interaction at the functional level can be suspected from the known biological functions of the newly expressed proteins.

The agronomic, phenotypic and compositional characteristics of soybean 305423 × 40-3-2 were compared under field conditions with those of the non-GM comparator (Jack) and three additional comparators (a negative segregant of soybean 305423 × 40-3-2 and the single-event parental lines soybean 305423 and 40-3-2), and tested for equivalence with a set of non-GM soybean reference varieties. Differences were identified in soybean 305423 × 40-3-2 with respect to the comparators for some agronomic endpoints, but these did not give rise to any food and feed or environmental safety concern. Except for the altered fatty acid profile (consistent with the intended trait), no food/feed safety assessment was needed for the compositional differences identified between soybean 305423 × 40-3-2 and the comparators, or for any lack of equivalence in composition to the non-GM soybean reference varieties. The comparison with the two parental lines did not reveal any potential interaction that could be of concern for food and feed safety.

No concerns were identified regarding the potential toxicity and allergenicity of proteins GM-HRA and CP4 EPSPS newly expressed in soybean 305423 × 40-3-2, and no evidence was found that the genetic modification might significantly change its overall allergenicity. Nutritional assessment of oil and other food products derived from soybean 305423 × 40-3-2 did not identify concerns for human health and nutrition. Based on the assessment of the single event 305423, on compositional data for soybean 305423 × 40-3-2 and on the results of a feeding study in chickens for fattening, there are no concerns regarding the use of feedingstuffs derived from defatted toasted 305423 × 40-3-2 soybean meal.

Considering the intended, altered nutritional composition of soybean 305423 × 40-3-2, a proposal for a post-market monitoring (PMM) plan needs to be provided by the applicant.

Application EFSA-GMO-NL-2007-47 covers the import, processing, and food and feed uses of soybean 305423 × 40-3-2, and excludes cultivation. Therefore, the ERA is concerned with the accidental release into the environment of viable soybean 305423 × 40-3-2 seeds (i.e. during transport and/or processing), and with the exposure to recombinant DNA of bacteria in the gastrointestinal tract of animals fed GM material and bacteria present in environments exposed to their faecal material (manure and faeces).

In the case of accidental release into the environment of viable seeds of soybean 305423 × 40-3-2, there are no indications of an increased likelihood of establishment and spread of occasional feral soybean 305423 × 40-3-2 plants, unless these plants are exposed to acetolactate-synthase (ALS)-inhibiting or glyphosate-containing herbicides. Considering the scope of the application EFSA-GMO-NL-2007-47, interactions with the biotic and abiotic environment are not considered to be relevant issues. Risks associated with an unlikely, but theoretically possible, horizontal gene transfer from soybean 305423 × 40-3-2 to bacteria have not been identified.

Therefore, considering the introduced traits, the outcome of the comparative analysis, the routes of exposure and the limited exposure levels, the GMO Panel concludes that soybean 305423 × 40-3-2 would not raise safety concerns in the event of accidental release of viable GM soybean seeds into the environment.

The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of soybean 305423 × 40-3-2 and the GMO Panel guidelines on the PMEM of GM plants.

In delivering its scientific opinion, the GMO Panel took into account application EFSA-GMO- NL-2007-47, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications. In conclusion, the GMO Panel is of the opinion that the two-event stack soybean 305423 × 40-3-2, as described in this application, is as safe as the non-GM comparator and the non-GM commercial soybean varieties with respect to potential effects on human and animal health and the environment in the context of its scope.

As already stated in the frame of the analysis of event 305423, considering the modified nutritional composition of soybean 305423 × 40-3-2, the 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 considers that this proposal is consistent with the compositional data provided for this soybean.

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1. Introduction

1.1. Background

On 24 September 2007, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands application EFSA-GMO-NL-2007-47, for authorisation of genetically modified (GM) soybean 305423 × 40-3-2 for food and feed uses, import and processing submitted by Pioneer within the framework of Regulation (EC) No 1829/2003[1] on genetically modified food and feed.

After receiving the application EFSA-GMO-NL-2007-47 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 available to the public on the EFSA website.[2] 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 20 November 2007, EFSA received additional information (requested on 12 November 2007). On 19 February 2008, EFSA declared the application valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to the Member States and the European Commission, and consulted nominated risk assessment bodies of the Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC[3] following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003 to request their scientific opinion. The Member States had 3 months after the opening of the Member State commenting period (until 19 May 2008) to make their opinion known.

The Panel on Genetically Modified Organisms of the European Food Safety Authority (GMO Panel) carried out an evaluation of the scientific risk assessment of soybean 305423 × 40-3-2 for food and feed uses, import and processing. The GMO Panel took into account the appropriate principles described in its guidelines for the risk assessment of GM plants and derived food and feed (EFSA, 2006, 2007; EFSA GMO Panel, 2011a), the environmental risk assessment (ERA) of GM plants (EFSA GMO Panel, 2010a) and on the post-market environmental monitoring (PMEM) of GM plants (EFSA GMO Panel, 2011b). Additional information received after May 2011 was assessed in accordance with 2011 guidance (EFSA GMO Panel, 2011a). Furthermore, the GMO Panel also took into consideration the scientific comments of the Member States, the additional information provided by the applicant and relevant scientific publications.

On 8 December 2009, 13 February 2014, 1 April 2014, 22 May 2014, 4 July 2014, 25 July 2014, 4 September 2014, 10 November 2014, 10 February 2015, 21 April 2015, 5 May 2015, 10 September 2015, 8 December 2015 and 21 April 2016, the GMO Panel requested additional information from the applicant. The applicant provided the requested information on 27 January 2010, 26 February 2014, 22 April 2014, 20 June 2014, 3 July 2014, 24 July 2014, 9 September 2014, 6 October 2014, 1 December 2014, 16 April 2015, 13 May 2015, 29 June 2015, 25 September 2015, 1 February 2016 and on 19 May 2016. The applicant also provided spontaneously additional information on 5 December 2013, 30 July 2015 and on 9 November 2015. The clock of the application was stopped on 29 February 2008 and maintained stopped from 15 December 2010 to 10 December 2013 due to the pending assessment of the single-event soybean 305423 (application reference EFSA-GMO-NL-2007-45). The applicant provided clarifications on the Labelling proposal of the application on 3 September 2015.

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

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

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.
[2] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2007-175
[3] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 12.3.2001, p. 1–38.

1.2. Terms of Reference as provided by the requestor

The GMO Panel was requested to carry out a scientific assessment of soybean 305423 × 40-3-2 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 (PMM) 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 GMO Panel was not requested to give an opinion on information required under Annex II to the Cartagena Protocol. The GMO Panel did consider if there is a need for 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.

2. Data and methodologies

2.1. Data

In delivering its scientific opinion, the GMO Panel took into account application EFSA-GMO-NL-2007-47, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications.

2.2. Methodologies

The GMO Panel carried out a scientific risk assessment of soybean 305423 × 40-3-2 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. The GMO Panel took into account the appropriate principles described in its guidelines for the risk assessment of GM plants and derived food and feed (EFSA, 2006, 2007; EFSA GMO Panel, 2011a), for the ERA of GM plants (EFSA GMO Panel, 2010a) and for the PMEM of GM plants (EFSA GMO Panel, 2011b).

The comments raised by the Member States are addressed in Annex G of EFSA's overall opinion[4] and were taken into consideration during the scientific risk assessment.

3. Assessment

3.1. Introduction

Application EFSA-GMO-NL-2007-47 covers a two-event stack soybean 305423 × 40-3-2 produced by conventional crossing. The scope of this application is for food and feed uses, import and processing, but excludes cultivation in the European Union (EU).

Soybean 305423 × 40-3-2 was developed to confer tolerance to acetolactate-synthase (ALS)-inhibiting herbicides and glyphosate (*N*-(phosphonomethyl)glycine)-based herbicides and to have an altered fatty acid profile (increased oleic acid content). Tolerance to ALS-inhibiting herbicides is conferred by the expression of the *Glycine max* herbicide-resistant ALS (GM-HRA) protein; tolerance to glyphosate is achieved by expression of the CP4 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS). The high-oleic acid phenotype is achieved by introducing a fragment of the soybean *fad2-1* gene, under the control of a promoter driving expression mainly in the seeds. The genetic modification results in the suppression of the expression of the endogenous omega-6 desaturase via RNA interference (RNAi).

The two single soybean events 305423 and 40-3-2 have been previously assessed (Table 1) and no concerns for human and animal health or environmental safety were identified.

[4] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2016-00198

Table 1: Single soybean events already assessed by the EFSA Panel on Genetically Modified Organisms (GMO Panel)

Event	Application or mandate	EFSA Scientific Opinion
305423	EFSA-GMO-NL-2007-45	EFSA GMO Panel (2013)
40-3-2	EFSA-GMO-NL-2005-24 EFSA-GMO-RX-40-3-2	EFSA GMO Panel (2012a) EFSA GMO Panel (2010b)

The EFSA guidance applicable to this application establishes that 'Where all single events have been assessed, the risk assessment of stacked events should focus mainly on issues related to (a) stability, (b) expression of the events and (c) potential interactions between the events' (EFSA, 2007). Additional information received after May 2011 was assessed in accordance with 2011 guidance (EFSA GMO Panel, 2011a).

3.2. Updated information on single events

Since the publication of the scientific opinions on the single soybean events by the GMO Panel (EFSA GMO Panel, 2010b, 2012a, 2013), no safety issue pertaining to the two single events has been reported by the applicant.

Updated bioinformatic analyses on the junction regions for events 305423 and 40-3-2 confirmed that no known endogenous genes were disrupted by any of the inserts.[5] Updated bioinformatic analyses of the amino acid sequence of the newly expressed proteins revealed no significant similarities to known toxins.[5] An updated search for similarity of the newly expressed proteins to allergens was performed applying the criterion of > 35% identity in an 80 amino acid sliding window.[5] This analysis did not reveal any new information regarding the similarity of GM-HRA and CP4 EPSPS proteins to known allergens. In addition, updated bioinformatic analyses of the newly created open reading frames (ORFs) within the inserts and at their junctions indicate that the expression of an ORF showing significant similarities to toxins or allergens is highly unlikely.[5]

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

3.3. Molecular characterisation

Possible interactions affecting the integrity of the single events, protein expression levels or the biological function conferred by the individual inserts are considered.

3.3.1. Genetic elements and biological functions of the inserts

The two single events 305423 and 40-3-2 are combined by conventional crossing to produce the two-event stack soybean 305423 × 40-3-2. The structures of the inserts introduced into the two-event stack soybean are described in detail in the respective EFSA scientific opinions (Table 1) and no new genetic modifications were involved. Genetic elements in the expression cassettes of the single events are summarised in Table 2.

Table 2: Genetic elements in the expression cassettes of the events stacked in soybean 305423 × 40-3-2

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
305423	KTi3 (*Glycine max*)	–	–	*fad2-1* (non-coding fragment) (*G. max*)	KTi3 (*G. max*)
	S-adenosyl-L-methionine synthetase (SAMS) (*G. max*)	5′ UTR and intron from SAMS (*G. max*)	–	*gm-hra* (*G. max*)	als (*G. max*)

[5] Additional information: 16/4/2015.

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
40-3-2	35S (CaMV)	–	CTP4 (*Petunia hybrida*)	CP4 *epsps* (*Agrobacterium tumefaciens*)	nos (*A. tumefaciens*)

CAMV: cauliflower mosaic virus; UTR: untranslated region; CTP4: chloroplast transit peptide 4; *fad*: fatty acid desaturase; *gm-hra*: *Glycine max* herbicide-resistant ALS; *epsps*: 5-enolpyruvylshikimate-3-phosphate synthase; KTi3: Kunitz trypsin inhibitor gene 3; als: acetolactate synthase; nos: nopaline synthase.
–: no element was specifically introduced to optimise expression

Intended effects of the inserts in soybean 305423 × 40-3-2 are summarised in Table 3.

Table 3: Characteristics and intended effects of the events stacked in soybean 305423 × 40-3-2

Event	Protein	Donor organism and biological function	Intended effects in GM plant
305423	FAD2-1[(a)]	Donor organism: *Glycine max*. The full-length FAD2-1 protein is an omega-6 desaturase in soybean. Its expression is seed-specific and the enzyme is responsible for the synthesis of the polyunsaturated fatty acids found in soybean oil (Heppard et al., 1996).	The inserted fragment of *fad2-1* does not code for a functional protein, rather suppresses the expression of the endogenous omega-6 desaturase by RNAi, resulting in an increased oleic acid phenotype
	GM-HRA	Donor organism: *G. max* Acetohydroxyacid synthase (AHAS or ALS) is a key enzyme that catalyses the first common step in the biosynthesis of the essential branched-chain amino acids isoleucine, leucine and valine (Coruzzi and Last, 2000; Duggleby and Pang, 2000; LaRossa and Falco, 1984; LaRossa and Schloss, 1984)	GM-HRA is a modified version of the endogenous ALS enzyme that confers tolerance to ALS-inhibiting herbicides (Hartnett et al., 1990)
40-3-2	CP4 EPSPS	Donor organism: *Agrobacterium* sp. 5-enopyruvyl-shikimate-3-phospate synthase (EPSPS) synthase is an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Herrmann, 1995)	The bacterial CP4 EPSPS confers tolerance to glyphosate-containing herbicides, as it has a greatly reduced affinity towards glyphosate as compared to the plant endogenous enzyme

FAD: Fatty Acid Desaturase; GM-HRA: *Glycine max* herbicide-resistant ALS.
(a): Gene fragment of the endogenous omega-6 desaturase.

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

3.3.2. Integrity of the events in soybean 305423 × 40-3-2

The genetic stability of the inserted DNA over multiple generations in the single soybean events 305423 and 40-3-2 was demonstrated previously (EFSA GMO Panel, 2010b, 2012a, 2013). Integrity of these events was demonstrated by Southern analyses.[6,7] Additional real-time polymerase chain reaction data was provided demonstrating the presence of all the inserts deriving from soybean 305423 in the two-event stack.[7]

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

Plants were grown in 2005 (six locations, three replicate plots) and in 2011 (10 locations, four replicate plots) under field conditions in the USA and in Canada.[9,10] The levels of GM-HRA and CP4 EPSPS proteins in the two-event stack soybean and the two single events were quantified by enzyme-linked immunosorbent assay (ELISA). Protein levels were determined in leaves (V2, V5, R1 (only in 2011) and R3 stages), forage (R3 stage), roots (R3 stage) and in seeds (R8 stage). Data on seeds are

[6] Dossier: Part I – Section D2.
[7] Additional information: 25/9/2015.
[8] Dossier: Part I – Section D3.
[9] Dossier: Part I – Annex 4.
[10] Additional information: 5/12/2013 – Annex 3.

reported in Table 4. GM-HRA and CP4 EPSPS protein levels in the two-event stack soybean were similar to the corresponding levels in the single soybean events and showed no major changes that could be the results of an interaction between the events.

Table 4: Means, standard deviations and ranges (n = 18 and n = 40 for 2005 and 2011 values, respectively) of protein levels in seeds (μg/g dry weight) from soybean 305423 × 40-3-2 and from single soybean events 305423 and 40-3-2

Tissue/protein	305423 × 40-3-2		305423		40-3-2	
	Untreated[a]	Treated[b]	Untreated	Treated	Untreated	Treated
Grain (2005)						
GM-HRA	3.1[c] ± 0.85[d] (1.9–4.6)[e]	2.7 ± 1.0 (1.8–5.9)	2.5 ± 1.1 (0–4.9)	2.5 ± 0.54 (1.7–3.5)	–	–
CP4 EPSPS	410 ± 70 (320–520)	400 ± 70 (310–550)	–	–	320 ± 50 (220–430)	340 ± 70 (230–500)
Grain (2011)						
GM-HRA	3.1 ± 0.67 (1.2–4.8)	2.9 ± 0.51 (2.1–4.0)	4.6 ± 0.78 (3.1–6.6)	4.4 ± 1.3 (< 0.54–7.2)	–	–
CP4 EPSPS	770 ± 280 (340–1,400)	770 ± 310 (200–1,800)	–	–	500 ± 190 (280–1,300)	460 ± 140 (280–840)

–: not assayed.
(a): Untreated: Plants were treated with conventional herbicides (quizalofop *p*-ethyl- and fomesafen-containing herbicides).
(b): Treated: Plants were treated with intended herbicides (ALS-inhibiting (chlorimuron and thifensulfuron) and/or glyphosate-containing herbicides).
(c): Mean.
(d): Standard deviation.
(e): Range.

In addition to protein expression analyses, the presence of the high-oleic acid phenotype and herbicide tolerance in soybean 305423 × 40-3-2 was also demonstrated by Northern analysis of the *fad2-1* gene transcript, gas chromatography and lateral-flow test for the CP4 EPSPS protein.[11]

3.3.4. Conclusion

The molecular data establish that the events stacked in soybean 305423 × 40-3-2 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins are similar to the corresponding levels in the single soybean events. Therefore, there is no indication of interaction that may affect the integrity of the events and the levels of the newly expressed proteins in this stack.

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

3.4. Comparative analyses

3.4.1. Choice of comparator and production of material for the comparative assessment[12]

In the context of this application, the applicant submitted data from two different field trials studies (Table 5).

[11] Dossier: Part I – Annex 2, 3 and 5.
[12] Dossier: Part I – Annex 4; additional information: 5/12/2013, 22/4/2014, 20/6/2014, 25/7/2014, 6/10/2014, 1/12/2014, 29/6/2015, 9/11/2015, 1/2/2016 and 19/5/2016.

Table 5: Overview of the field trial studies for the comparative assessment of agronomic and phenotypic characteristics and composition of soybean 305423 × 40-3-2

Study details	Comparators			Non-GM commercial reference varieties
	Non-GM comparator	Negative segregant	Parental lines	
USA and Canada, 2005, six locations	–	BC_1F_5	–	–
USA and Canada, 2011, 10 locations	Jack	BC_1F_7	40-3-2 and 305423	Ten varieties

GM: Genetically modified.
–: The material was not grown in the field trials.

In the 2005 field trials,[9] soybean 305423 × 40-3-2 was compared to a negative segregant line (generation BC_1F_5) not containing the events 40-3-2 and 305423. The GMO Panel is of the opinion that potential unintended effects linked to the genetic modification process in the GM plant cannot be identified using a negative segregant as the sole comparator (EFSA GMO Panel, 2011a,c). Therefore, the GMO Panel considers that the field trials performed in 2005 are not appropriate.

The 2011 field trials were performed at ten sites in the major soybean growing regions of North America.[13] The materials were treated either with conventional herbicides[14] (CHT) or with the intended herbicides[15] (IHT). At each site, the following materials were grown: soybean 305423 × 40-3-2 (CHT), soybean 305423 × 40-3-2 (IHT), the non-GM soybean variety Jack (CHT), a negative segregant of 305423 × 40-3-2 (CHT), the parental line single-event soybean 305423 (CHT and IHT), the parental line single-event soybean 40-3-2 (CHT and IHT) and three commercial non-GM soybean reference varieties (CHT; out of ten varieties in total[16]). A randomised complete block design with four replicates of each material was used at each site. The material harvested in these field trials was used for compositional analyses of forage and seeds.

In the first analysis of the 2011 field trials,[17] soybean 305423 × 40-3-2 was compared to the non-GM soybean variety Jack (Table 5). On the basis of the breeding diagram and the estimated genetic similarity with the stack, the GMO Panel concluded that Jack is a suboptimal comparator, and that it cannot be used as the *sole* comparator for the agronomic, phenotypic and compositional characterisation of soybean 305423 × 40-3-2.

In order to address the limitations related to the choice of Jack, the GMO Panel asked the applicant to provide the complete data set from the 2011 field trials, which included materials that could be used as additional comparators. These additional comparators were: the selected negative segregant of soybean 305423 × 40-3-2 (generation BC_1F_7); the parental line single-event soybean 305423 (CHT); and the parental line single-event soybean 40-3-2 (CHT). The use of additional comparators, including parental lines and negative segregants, is foreseen if deemed useful to support the risk assessment (EFSA GMO Panel 2011a,c). The GMO Panel based its assessment on the analysis carried out on the complete data set.[18]

The statistical analysis of agronomic, phenotypic and compositional data from the 2011 field trials was carried out in line with the applicable EFSA Guidance (EFSA GMO Panel, 2011a). This includes the application of a test of difference and a test of equivalence to each endpoint.

- The test of difference determines whether or not the GM plant is different from its comparator. In the analysis of the 2011 field trials, the test of difference was performed between soybean 305423 × 40-3-2 (CHT and IHT) and each of the four comparators:
  - Jack;
  - the negative segregant;
  - soybean 305423 (CHT);
  - soybean 40-3-2 (CHT).

[13] Nine sites were in the USA: Iowa (two sites), Illinois (two sites), Kansas, Minnesota, Nevada, Pennsylvania and Wisconsin. One site was in Canada (Ontario).
[14] The conventional herbicides were a tank mix of herbicides with the active ingredients quizalofop *p*-ethyl and fomesafen.
[15] The intended herbicides were: chlorimuron, thifensulfuron, and glyphosate for soybean 305423 × 40-3-2; chlorimuron and thifensulfuron for soybean 305423; glyphosate for soybean 40-3-2.
[16] The commercial non-GM soybean reference materials were the Pioneer brand® lines 92M10, 92M22, 92M72, 92Y21, 93B82, 93M14, 93M52, 93M62, 93Y21 and 93Y41.
[17] Additional information: 5/12/2013.
[18] Additional information: 1/2/2016 and 19/5/2016.

- The test of equivalence determines whether or not the GM plant (here, soybean 305423 × 40-3-2 CHT and IHT) falls within the range of natural variation estimated from the non-GM soybean reference varieties. The results of the equivalence test are categorised into four possible outcomes (I–IV, from equivalence to non-equivalence).[19]

In the rest of this Section, the herbicide treatment (CHT/IHT) will be explicitly indicated only for soybean 305423 × 40-3-2; for all the other materials that will be discussed, it is specified here that the herbicide treatment is CHT.

Regarding the test of difference, the GMO Panel assessed the results of the four sets of comparisons as follows:

- The GMO Panel considered that the use of a negative segregant as additional comparator is a suitable way to address the limitations related to the choice of Jack. Therefore, the results of the comparison of soybean 305423 × 40-3-2 with the negative segregant were assessed together with the results obtained with Jack. The comparison with the negative segregant may in general identify *additional* differences (not identified in the comparison with Jack) to be included in the risk assessment. The GMO Panel took the conservative approach of considering *all* the differences identified in the two comparisons, with no priority given to either comparator, as indicators of effects linked to the genetic modification. Every difference in the two sets of comparisons was therefore independently assessed.
- The results of the comparison of soybean 305423 × 40-3-2 with the two parental lines (single-events soybean 305423 and soybean 40-3-2) were used for the identification of differences that may be linked to interaction between the two single events. In addition, the comparison of soybean 305423 × 40-3-2 with the parental line soybean 305423 was used to confirm the effects of the genetic modification on the fatty acid profile.

3.4.2. Agronomic and phenotypic analysis[18]

Ten phenotypic and agronomic endpoints were measured at the 10 field trial sites in North America in 2011 (Table 5): early stand count, days to maturity, plant height, final stand count, pod shattering, yield, seedling vigour, lodging, disease incidence and insect damage. Nine of them were analysed with difference and equivalence testing (the remaining endpoint, pod shattering, did not fulfil the requirements of the statistical tests).

The results of the tests of difference were as follows:

- In the comparison with Jack, significant differences for soybean 305423 × 40-3-2 (both CHT and IHT) were identified for four endpoints: early population, plant height, lodging and final population; significant differences between Jack and soybean 305423 × 40-3-2 (CHT) were identified for two endpoints: days to maturity and yield.
- In the comparison with the negative segregant, significant differences for soybean 305423 × 40-3-2 (both CHT and IHT) were identified for four endpoints: early population, plant height, final population and yield; significant differences between the negative segregant and soybean 305423 × 40-3-2 (CHT) were identified for two endpoints: insect damage and lodging.
- In the comparison with soybean 305423, significant differences for soybean 305423 × 40-3-2 (both CHT and IHT) were identified for six endpoints: early population, insect damage, days to maturity, lodging, final population and plant height; a significant difference between soybean 305423 and soybean 305423 × 40-3-2 (IHT) was identified for the endpoint yield.
- In the comparison with soybean 40-3-2, significant differences for soybean 305423 × 40-3-2 (both CHT and IHT) were identified for six endpoints: early population, disease incidence, days to maturity, final population, lodging and plant height.

The test of equivalence[19] showed that all the endpoints analysed in 305423 × 40-3-2 soybean (both CHT and IHT) were equivalent to the non-GM soybean reference varieties (equivalence category I).

The GMO Panel concludes that taking all data together, the phenotypic and agronomic characteristics of soybean 305423 × 40-3-2 are comparable to the commercial non-GM soybean varieties, except for the introduced traits (tolerance to herbicides). The differences in agronomic and

[19] In detail, the four outcomes are: category I (indicating full equivalence); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence) (EFSA GMO Panel 2011a).

phenotypic characteristics identified between soybean 305423 × 40-3-2 and the comparators are further discussed for their potential environmental impact in Section 3.6.

3.4.3. Compositional analysis[18]

The seeds and forage of the soybean materials harvested from the field trials in North America in 2011 (Table 5) were analysed for 101 constituents (eight in forage and 93 in seeds), including the key constituents recommended for soybean by OECD (2001). Eighteen seed constituents[20] with 50% or more sample values below the lower limit of quantification were excluded from the comparative analysis, which therefore included 83 endpoints (eight in forage and 75 in seeds, of which 17 fatty acids).

The GMO Panel has already assessed data on the composition of soybean 305423 and soybean 40-3-2 (EFSA GMO Panel, 2010b, 2012a, 2013). The composition of soybean 40-3-2 was found equivalent to that of its conventional counterparts (A5403 and Dekabig) and commercial soybean varieties (EFSA GMO Panel, 2010b, 2012a). The composition of soybean 305423 was found different from the conventional counterpart (Jack) and commercial soybean varieties in having an altered fatty acid profile (EFSA GMO Panel, 2013).

In the following, a detailed discussion is provided of results for the fatty acid profile and, separately, for the other endpoints.

3.4.3.1. Results for the fatty acid profile

Statistical results (outcomes and estimates) for the 17 fatty acids[21] are reported in Table 6.

The outcomes of the test of difference for were the following:

- In the comparison with Jack, all the 17 fatty acids analysed were found significantly different from both treatments of soybean 305423 × 40-3-2 (CHT/IHT), with the exception of palmitoleic acid (C16:1).
- In the comparison with the negative segregant, all the 17 fatty acids analysed were found significantly different for soybean 305423 × 40-3-2 (both CHT and IHT).
- In the comparison with soybean 305423, 10 significant differences were identified for 305423 × 40-3-2 (CHT) and 11 significant differences for 305423 × 40-3-2 (IHT).
- In the comparison with soybean 40-3-2, all the 17 fatty acids analysed were found significantly different from soybean 305423 × 40-3-2 (both CHT and IHT).

The outcomes of test of equivalence[19] for the two herbicide treatments of soybean 305423 × 40-3-2 (CHT and IHT) were identical for each of the 17 endpoints. The level of three fatty acids in soybean 305423 × 40-3-2 fell under equivalence category I: palmitoleic acid (C16:1), stearic acid (C18:0) and (9,15) isomer of linoleic acid (C18:2). The level of the remaining 14 fatty acids in soybean 305423 × 40-3-2 fell under equivalence category III or IV.

[20] The endpoints excluded from the statistical analysis were sodium, genistein, glycitein, coumestrol and 14 fatty acids: caprylic (C8:0), capric (C10:0), lauric (C12:0), myristoleic (C14:1), pentadecanoic (C15:0), pentadecenoic (C15:1), heptadecadienoic (C17:2), γ-linolenic (C18:3), nonadecanoic (C19:0), isomer 1 of nonadecenoic (C19:1), eicosadienoic (C20:2), eicosatrienoic (C20:3), arachidonic (C20:4) and erucic (C22:1).

[21] The fatty acids analysed were: myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), heptadecanoic (C17:0), heptadecenoic (C17:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), (9,15) isomer of linoleic acid (C18:2), α-linolenic (C18:3), isomer 2 of nonadecenoic acid (C19:1), arachidic (C20:0), eicosenoic (C20:1), heneicosanoic (C21:0), behenic (C22:0), tricosanoic (C23:0) and lignoceric (C24:0).

Table 6: Statistical results for the 17 fatty acids: means (for the two-event stack soybean 305423 × 40-3-2 and the four comparators) and equivalence limits (from the non-GM reference varieties) estimated from the 2011 field trials (Table 5)

Fatty acids (% total FA)	Soybean 305423 × 40-3-2		Comparator				Equivalence limits from non-GM reference varieties
	CHT[a]	IHT[b]	Jack	Negative segregant	Soybean 305423	Soybean 40-3-2	
Saturated fatty acids							
Myristic acid (C14:0)	0.0472	0.0484	0.0675	0.0686	0.0482[c,d]	0.0727	(0.0595, 0.0843)
Palmitic acid (C16:0)	6.17	6.17	10.1	10.2	6.48	10.2	(9.81, 11.3)
Heptadecanoic acid (C17:0)	0.781	0.783	0.116	0.117	0.772[c]	0.114	(0.0996, 0.121)
Stearic acid (18:0)	4.35	4.40	4.17	4.64	4.17	5.25	(3.47, 5.07)
Arachidic acid (C20:0)	0.423	0.428	0.322	0.361	0.421[c]	0.410	(0.275, 0.399)
Heneicosanoic acid (C21:0)	0.0566	0.0639	0.0191	0.0205	0.0552[c,d]	0.0385	(0.0137, 0.0322)
Behenic acid (C22:0)	0.402	0.406	0.328	0.347	0.433	0.368	(0.288, 0.373)
Tricosanoic acid (C23:0)	0.0684	0.0688	0.0531	0.0598	0.0638	0.0605	(0.0417, 0.0678)
Lignoceric acid (C24:0)	0.175	0.180	0.146	0.150	0.202	0.149	(0.108, 0.174)
Monounsaturated fatty acids							
Palmitoleic acid (C16:1)	0.112	0.116	0.112[c]	0.106	0.122	0.122	(0.102, 0.144)
Heptadecenoic acid (C17:1)	1.23	1.23	0.0594	0.0542	1.24[c,d]	0.0591	(0.0534, 0.071)
Oleic acid (C18:1)	74.4	74.8	19.3	19.4	74.5[c,d]	21.3	(17, 22)
Nonadecenoic acid (C19:1) isomer 2	0.303	0.304	0.0497	0.0511	0.294[c,d]	0.0477	(0.0395, 0.0693)
Eicosenoic acid (C20:1)	0.362	0.361	0.174	0.183	0.353	0.188	(0.164, 0.206)
***n*-3 polyunsaturated fatty acids**							
Isomer (9,15) of linoleic acid (C18:2)	0.721	0.752	0.642	0.62	0.766[d]	0.658	(0.499, 0.915)
α-Linolenic acid (C18:3)	5.26	5.18	8.17	8.79	4.94	8.22	(6.97, 9.58)
***n*-6 polyunsaturated fatty acids**							
Linoleic acid (C18:2)	4.35	4.17	50.0	48.9	4.83	47.0	(36.8, 77.5)

The outcomes of the difference test are shown in the entries for the comparators: entries that are *not significantly different* from the stack (CHT, IHT or both) are marked with upper indices[c],[d],[c,d]; for entries with *no indices*, the stack (CHT/IHT) is significantly different from the comparator. The outcomes of the equivalence test are shown in the entries for the stack, and are differentiated by greyscale backgrounds: white (equivalence category I), light grey (equivalence category III) and dark grey (equivalence category IV).
(a): Treated with conventional herbicides (quizalofop *p*-ethyl and fomesafen).
(b): Treated with the intended herbicides (chlorimuron, thifensulfuron and glyphosate).
(c): No significant difference identified between 305423 × 40-3-2 (CHT) and the comparator.
(d): No significant difference identified between 305423 × 40-3-2 (IHT) and the comparator.
(c,d): No significant differences identified between 305423 × 40-3-2 (both CHT and IHT) and the comparator.

In interpreting the results in Table 6, the two comparisons with Jack and the negative segregant are considered first, followed by the comparisons with soybean 305423 and soybean 40-3-2.

Several of the significant differences identified in the comparison of soybean 305423 × 40-3-2 with Jack and with the negative segregant are consistent with the intended effect of the genetic modification characterising event 305423 (EFSA GMO Panel, 2013): an increase in oleic acid (C18:1) at the expense of the polyunsaturated fatty acids linoleic (18:2) and α-linolenic (18:3). Further changes associated to the intended effect are the increase in eicosenoic acid (20:1) and the decrease in myristic acid (14:0) and palmitic acid (16:0). All these endpoints fell under equivalence category IV. Another set of significant differences corresponds to an expected unintended effect associated to soybean event 305423 (EFSA GMO Panel, 2013): an increase in the levels of odd-chain fatty acids (e.g. heptadecanoic acid (C17:0) and heptadecenoic acid (C17:1)); all these endpoints fell under equivalence categories III or IV. An explanation considered plausible by the GMO Panel is that the GM-HRA enzyme may have a decreased affinity for 2-ketobutyrate. This may lead to an increased pool of 2-ketobutyrate available for odd chain fatty acid biosynthesis, hence to increased levels of odd chain fatty acids in soybean 305423 × 40-3-2 (EFSA GMO Panel, 2013).

The results of the comparison of soybean 305423 × 40-3-2 with soybean 305423 show that the intended and expected unintended effects linked to event 305423 occur consistently in the single event and in the two-event stack. Of the endpoints associated to the two effects, several were found not significantly different between soybean 305423 × 40-3-2 (CHT/IHT) and soybean 305423 (e.g. oleic acid (18:1), heptadecenoic acid (C17:1)). Several other endpoints were significantly different (e.g. linoleic acid (18:2)); however, in all those cases, the levels in soybean 305423 × 40-3-2 were much closer to soybean 305423 than to Jack or the negative segregant. Overall, this confirms that the pattern of the fatty acid profile is maintained in the two-event stack.

The results of the comparison of soybean 305423 × 40-3-2 with the two single parental lines did not provide evidence of an impact of the combination of the two events 305423 and 40-3-2 on the overall fatty acid profile of 305423 × 40-3-2.

The changed fatty acid profile of 305423 × 40-3-2 is assessed for possible nutritional and safety implications in Section 3.5.5.

3.4.3.2. Results for the other compounds

The outcome of the test of difference for compounds other than fatty acids (eight in forage and 58 in seed[22]) was the following:

- In the comparison with Jack, significant differences with soybean 305423 × 40-3-2 (CHT) were identified for 42 compounds (40 in seed and two in forage); significant differences with soybean 305423 × 40-3-2 (IHT) were identified for 45 compounds (41 in seed and four in forage).[23]
- In the comparison with the negative segregant, significant differences with soybean 305423 × 40-3-2 (CHT) were identified for 42 compounds (38 in seed and four in forage);

[22] Endpoints in forage: ash, carbohydrates, moisture, crude protein, crude fat, crude fibre, acid detergent fibre (ADF), neutral detergent fibre (NDF). Endpoints in seed: proximates and fibres (ash, carbohydrates, moisture, crude protein, crude fat, crude fibre, ADF, NDF), 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, sodium, zinc), vitamins (vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 (niacin), vitamin B5 (pantothenic acid), vitamin B6 (pyridoxine), vitamin B9 (folic acid), α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol, total tocopherols), isoflavones (daidzein, daidzin, genistein, genistin, glycitein, glycitin, total daidzein, total genistein, total glycitein), oligosaccharides (raffinose, stachyose, sucrose), secondary metabolites and antinutrients (coumestrol, lectins, phytic acid, trypsin inhibitor).

[23] Significantly different forage endpoints identified in the comparison between soybean 305423 × 40-3-2 and Jack: ADF (for soybean 305423 × 40-3-2 CHT and IHT), ash (CHT, IHT), crude fibre (IHT) and NDF (IHT). Significantly different seed endpoints: ADF (IHT), carbohydrates (IHT), crude fat (CHT, IHT), crude fibre (CHT, IHT), alanine (CHT, IHT), aspartic acid (CHT, IHT), cystine (IHT), glutamic acid (IHT), glycine (CHT, IHT), isoleucine (CHT, IHT), leucine (CHT, IHT), methionine (IHT), phenylalanine (CHT, IHT), proline (CHT), serine (CHT), threonine (CHT, IHT), tryptophan (CHT, IHT), tyrosine (CHT, IHT), valine (CHT, IHT), calcium (CHT, IHT), copper (IHT), iron (CHT, IHT), manganese (CHT, IHT), potassium (CHT), zinc (CHT, IHT), α-tocopherol (CHT, IHT), δ-tocopherol (CHT, IHT), γ-tocopherol (CHT, IHT), total tocopherols (CHT, IHT), vitamin B1 (thiamine) (CHT, IHT), vitamin B2 (riboflavin) (CHT, IHT), vitamin B5 (pantothenic acid) (CHT, IHT), vitamin B6 (pyridoxine) (CHT, IHT), vitamin B9 (folic acid) (CHT, IHT), daidzein (CHT, IHT), daidzin (CHT, IHT), genistin (CHT), glycitin (CHT, IHT), total daidzein (CHT, IHT), total genistein (CHT), total glycitein (CHT, IHT), raffinose (CHT, IHT), stachyose (CHT, IHT), sucrose (CHT, IHT), lectins (CHT, IHT), trypsin inhibitor (CHT, IHT).

significant differences with soybean 305423 × 40-3-2 (IHT) were identified for 45 compounds (39 in seed and six in forage).[24]

- In the comparison with the parental line soybean 305423, significant differences with soybean 305423 × 40-3-2 (CHT) were identified for 40 compounds (34 in seed and six in forage); significant differences with soybean 305423 × 40-3-2 (IHT) were identified for 38 compounds (35 in seed and three in forage).[25]
- In the comparison with the parental line soybean 40-3-2, significant differences with soybean 305423 × 40-3-2 (CHT) were identified for 47 compounds (42 in seed and five in forage); significant differences with soybean 305423 × 40-3-2 (IHT) were identified for 33 compounds (31 in seed and two in forage).[26]

All the significant differences from the four comparisons were assessed; no issues were identified. The comparison of soybean 305423 × 40-3-2 with the two single parental lines, soybean 305423 and soybean 40-3-2, did not provide indication of interactions that would be of concern for food and feed safety or nutrition.

The test of equivalence[19] showed that for soybean 305423 × 40-3-2 (both CHT and IHT) 51 seed endpoints and seven forage endpoints fell under equivalence category I or II: these endpoints were not considered further. Six seed endpoints, which fell under equivalence category III or IV, and two endpoints for which the equivalence test could not be performed (because of the small variation among the non-GM reference varieties) were further considered (Table 7).

[24] Significantly different forage endpoints identified in the comparison between soybean 305423 × 40-3-2 and the negative segregant: ADF (for soybean 305423 × 40-3-2 IHT), ash (for soybean 305423 × 40-3-2 CHT and IHT), carbohydrates (CHT, IHT), crude fibre (IHT), crude protein (CHT), moisture (IHT), NDF (CHT, IHT). Significantly different seed endpoints: ADF (IHT), carbohydrates (CHT), crude fat (IHT), crude fibre (CHT, IHT), crude protein (CHT, IHT), NDF (CHT, IHT), alanine (CHT, IHT), aspartic acid (CHT, IHT), glutamic acid (CHT), glycine (CHT, IHT), histidine (IHT), isoleucine (CHT, IHT), leucine (CHT, IHT), lysine (CHT, IHT), phenylalanine (CHT, IHT), proline (CHT, IHT), serine (CHT, IHT), threonine (CHT, IHT), tryptophan (CHT, IHT), tyrosine (CHT, IHT), valine (CHT, IHT), calcium (CHT, IHT), magnesium (CHT, IHT), α-tocopherol (CHT, IHT), δ-tocopherol (CHT, IHT), γ-tocopherol (CHT, IHT), total tocopherols (CHT, IHT), vitamin B1 (thiamine) (CHT, IHT), vitamin B2 (riboflavin) (CHT, IHT), vitamin B3 (niacin) (CHT, IHT), vitamin B5 (pantothenic acid) (CHT, IHT), vitamin B6 (pyridoxine) (CHT, IHT), daidzein (CHT, IHT), genistin (CHT, IHT), glycitin (CHT, IHT), total genistein (CHT, IHT), total glycitein (CHT, IHT), stachyose (CHT, IHT), sucrose (CHT, IHT), lectins (CHT, IHT), trypsin inhibitor (CHT, IHT).

[25] Significantly different forage endpoints identified in the comparison between soybean 305423 × 40-3-2 and the parental line soybean 305423: ADF (for soybean 305423 × 40-3-2 CHT), ash (for soybean 305423 × 40-3-2 CHT and IHT), carbohydrates (CHT, IHT), crude fibre (CHT), crude protein (CHT), NDF (CHT, IHT). Significantly different seed endpoints: ash (CHT, IHT), carbohydrates (IHT), crude fat (IHT), moisture (CHT, IHT), NDF (CHT, IHT), glycine (CHT), histidine (CHT, IHT), leucine (CHT), methionine (IHT), threonine (CHT, IHT), valine (CHT, IHT), calcium (CHT, IHT), copper (CHT, IHT), iron (CHT, IHT), magnesium (CHT, IHT), manganese (CHT, IHT), phosphorus (CHT, IHT), potassium (CHT, IHT), zinc (CHT, IHT), δ-tocopherol (CHT, IHT), γ-tocopherol (CHT, IHT), total tocopherols (CHT, IHT), vitamin B2 (riboflavin) (CHT, IHT), vitamin B3 (niacin) (CHT, IHT), vitamin B5 (pantothenic acid) (CHT, IHT), vitamin B6 (pyridoxine) (CHT, IHT), vitamin B9 (folic acid) (CHT, IHT), daidzein (CHT, IHT), daidzin (CHT, IHT), genistin (CHT, IHT), glycitin (CHT, IHT), total daidzein (CHT, IHT), total genistein (CHT, IHT), total glycitein (CHT, IHT), raffinose (CHT, IHT), stachyose (CHT, IHT), trypsin inhibitor (CHT, IHT).

[26] Significantly different forage endpoints identified in the comparison between soybean 305423 × 40-3-2 and the parental line soybean 40-3-2: ADF (for soybean 305423 × 40-3-2 CHT), carbohydrates (for soybean 305423 × 40-3-2 CHT and IHT), crude fibre (CHT), crude protein (CHT, IHT), NDF (CHT). Significantly different seed endpoints: carbohydrates (CHT), crude fat (CHT), crude fibre (CHT, IHT), moisture (IHT), alanine (CHT), aspartic acid (CHT, IHT), glycine (CHT), histidine (CHT, IHT), isoleucine (CHT, IHT), leucine (CHT, IHT), lysine (CHT), phenylalanine (CHT), proline (CHT, IHT), threonine (CHT), tryptophan (CHT, IHT), tyrosine (CHT), valine (CHT, IHT), calcium (CHT, IHT), magnesium (CHT, IHT), phosphorus (CHT, IHT), potassium (CHT, IHT), zinc (CHT, IHT), α-tocopherol (CHT, IHT), β-tocopherol (CHT), δ-tocopherol (CHT, IHT), γ-tocopherol (CHT, IHT), total tocopherols (CHT, IHT), vitamin B1 (thiamine) (CHT, IHT), vitamin B2 (riboflavin) (CHT), vitamin B3 (niacin) (CHT), vitamin B5 (pantothenic acid) (CHT, IHT), vitamin B6 (pyridoxine) (CHT, IHT), daidzein (CHT, IHT), daidzin (CHT, IHT), genistin (CHT, IHT), glycitin (CHT, IHT), total daidzein (CHT, IHT), total genistein (CHT, IHT), total glycitein (CHT, IHT), stachyose (CHT, IHT), sucrose (CHT, IHT), phytic acid (CHT), trypsin inhibitor (CHT, IHT).

Table 7: Compositional endpoints that are further assessed based on the results of the statistical analysis: means (for the two-event stack soybean 305423 × 40-3-2 and the four comparators) and equivalence limits (from the non-GM reference varieties) estimated from the 2011 field trials (Table 5)

Endpoint	Soybean 305423 × 40-3-2		Comparator				Equivalence limits from non-GM reference varieties
	CHT[(a)]	IHT[(b)]	Jack	Negative segregant	Soybean 305423	Soybean 40-3-2	
Seed constituents							
ADF (% dw)	15.7	15.9	15.4[(c)]	15.3[(c)]	15.9[(c,d)]	16.2[(c,d)]	Not applied
Calcium (mg/kg dw)	2,260	2,250	2,560	2,580	2,360	2,400	(2,320, 2,740)
Zinc (mg/kg dw)	51.7	51.6	54.2	50.6	57.1[(c,d)]	49.5	(41.6, 49.5)
Riboflavin (mg/kg dw)	3.95	3.87	3.56	3.55	3.70	3.80[(d)]	(3.14, 3.82)
Glycitin (mg/kg dw)	283	289	310	208	423	166	(143, 280)
Total glycitein (mg/kg dw)	183	187	200	135	272	108	(93.4, 181)
Trypsin inhibitor (TIU/mg dw)	18.1	18.7	29.4	30.8	13.8	30.8	(26.2, 38.7)
Forage constituents							
Crude fibre (% dw)	30.2	29.2	30.3[(c)]	30.4[(c)]	28.8[(d)]	29[(d)]	Not applied

The outcome of the test of difference is shown in the entries for the comparators: entries that are *not significantly different* from the stack (CHT, IHT or both) are marked with upper indices[(c),(d),(c,d)]; for entries with *no indices*, the stack (CHT/IHT) is significantly different from the comparator. The outcome of the test of equivalence is shown in the entries for the stack, and it is differentiated by greyscale backgrounds: white (the equivalence test was not performed), light grey (equivalence category III) and dark grey (equivalence category IV).
dw: dry weight; TIU: trypsin inhibitor units.
(a): Treated with conventional herbicides (quizalofop *p*-ethyl and fomesafen).
(b): Treated with the intended herbicides (chlorimuron, thifensulfuron and glyphosate).
(c): No significant difference identified between 305423 × 40-3-2 (CHT) and the comparator.
(d): No significant difference identified between 305423 × 40-3-2 (IHT) and the comparator.
(c,d): No significant differences identified between 305423 × 40-3-2 (both CHT and IHT) and the comparator.

The level of trypsin inhibitor in soybean 305423 × 40-3-2 (CHT and IHT) fell under equivalence category IV and was significantly different (lower) than in Jack and the negative segregant (Table 7). The results for trypsin inhibitor are consistent with the expected unintended effect that was already observed for soybean event 305423 (EFSA GMO Panel, 2013). The decrease is caused by silencing of the endogenous *KTi3* gene (encoding a Kunitz trypsin inhibitor), and is therefore also expected to occur in the context of the stack. A decreased level of trypsin inhibitor is not posing any food and feed safety concern.

The GMO Panel assessed all the significant differences, and any lack of equivalence, for the remaining endpoints in Table 7. After considering their well-known biological role and the magnitudes of the changes observed, the GMO Panel did not identify any need for further food/feed safety assessment.

3.4.4. Conclusion

The GMO Panel concludes that soybean 305423 × 40-3-2 differs from the non-GM comparator Jack and the negative segregant, and is not equivalent to the non-GM soybean reference varieties, in having an altered fatty acid profile. The changes in fatty acid profile are consistent with those observed in the single-event soybean 305423, including the increase in oleic acid content (the intended trait). The altered fatty acid profile is assessed in Section 3.5.5. No further assessment

for food and feed safety was needed for the other differences, or any other lack of equivalence, identified in the composition and in the agronomic and phenotypic characteristics of soybean 305423 × 40-3-2. The comparison with the two parental lines did not reveal any potential interaction that could be of concern for food and feed safety.

The differences in agronomic and phenotypic characteristics identified between soybean 305423 × 40-3-2 and the four comparators are further discussed for their potential environmental impact in Section 3.6.

3.5. Food and feed safety assessment

3.5.1. Effect of processing[27]

Soybean 305423 × 40-3-2 will undergo the existing methods of production and processing used for commercial soybean. No novel methods of production and processing are envisaged.

Seeds of soybean 305423 × 40-3-2 collected from the 2011 field trials (Table 5) were processed into refined bleached deodorised (RBD) oil and analysed for fatty acid composition.[28] The GMO Panel concluded that the fatty acid profile of RBD oil obtained from soybean 305423 × 40-3-2 was similar to the fatty acid profile of unprocessed seeds (Table 8). This was also previously noted for soybean 305423 (EFSA GMO Panel, 2013).

Table 8: Mean values of fatty acid levels in seed oil and RBD oil[29] from 305423 × 40-3-2

	Soybean 305423 × 40-3-2 (IHT[(a)])	
Fatty acids (% total FA)	**Seed oil[(b)]**	**RBD oil[(c)]**
Saturated fatty acids		
Myristic acid (C14:0)	0.0484	0.00
Palmitic acid (C16:0)	6.17	6.20
Heptadecanoic acid (C17:0)	0.783	0.75
Stearic acid (18:0)	4.40	3.88
Arachidic acid (C20:0)	0.428	0.41
Heneicosanoic acid (C21:0)	0.0639	0.00
Behenic acid (C22:0)	0.406	0.46
Tricosanoic acid (C23:0)	0.0688	0.00
Lignoceric acid (C24:0)	0.180	0.202
Monounsaturated fatty acids		
Palmitoleic acid (C16:1)	0.116	0.10
Heptadecenoic acid (C17:1)	1.23	1.35
Oleic acid (C18:1)	74.8	74.7
Nonadecenoic acid (C19:1) isomer 1	0	0.36
Nonadecenoic acid (C19:1) isomer 2	0.304	0.31
Eicosenoic acid (C20:1)	0.361	0.38
n-3 polyunsaturated fatty acids		
(9,15) isomer of linoleic acid (C18:2)	0.752	0.645
α-Linolenic acid (C18:3)	5.18	4.96
n-6 polyunsaturated fatty acids		
Linoleic acid (C18:2)	4.17	3.76

RBD: refined bleached deodorised; FA: fatty acid.
(a): Treated with the intended herbicides (chlorimuron, thifensulfuron and glyphosate).
(b): Mean values extracted from (Table 6).
(c): Average (rounded) of the values for two pooled samples of RBD oil. The two samples were produced combining seeds from two non-overlapping sets of five trials sites.

[27] Dossier: Part I – Section A3.5.
[28] Additional information: 9/9/2014 and 29/6/2015.
[29] Additional information: 29/6/2015.

3.5.2. Toxicology

3.5.2.1. Toxicological assessment of newly expressed proteins

Two proteins (GM-HRA and CP4 EPSPS) are newly expressed in the two-event stack soybean 305423 × 40-3-2.

The GMO Panel has previously assessed these proteins in the context of the single events (Table 1), as well as in other soybean events (EFSA GMO Panel 2011d, 2012b) and no safety concern for humans or animals was identified. The GMO Panel is not aware of any new information that would change these conclusions. Updated bioinformatic studies[5] confirmed the absence of relevant similarities between these newly expressed proteins to known toxins.

The potential for a functional interaction of these newly expressed proteins in two-event stack soybean 305423 × 40-3-2 has been assessed with regard to human and animal health. The two proteins are enzymes which catalyse distinct biochemical reactions and act on unrelated substrates in the plant. No reasons were identified to expect that the presence of the two proteins in combination would result in interactions producing effects different from those of the individual proteins (Section 3.3). As the individual proteins are considered safe for humans and animals, the same conclusion can be extended to their presence in the two-event stack soybean 305423 × 40-3-2.

The GMO Panel concludes that there are no safety concerns to human and animal health related to GM-HRA and CP4 EPSPS proteins newly expressed in soybean 305423 × 40-3-2.

3.5.2.2. Toxicological assessment of components other than newly expressed proteins

The compositional analysis of soybean 305423 × 40-3-2 confirmed the expected altered fatty acid profile in seeds and oil (Table 8). All of these fatty acids occur naturally in the diet of humans and animals. The safety impact of the altered fatty acid profile of soybean 305423 × 40-3-2 is evaluated in Section 3.5.5.

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

3.5.3.1. Subchronic feeding study in rats[5]

The applicant provided a publication (Qi et al., 2012) describing a subchronic study in rats fed diets containing up to 30% raw flour from soybean 305423 × 40-3-2 or from a non-GM soybean. An additional group was fed a commercial diet not containing soybean flour or meal. Diets are reported to be nutritionally balanced (Chinese standard NY/T1102-2006). The GMO Panel notes that limited information is provided on the study design, material and methods and results, as well as on its GLP compliance status. The GMO Panel also notes the low number of experimental units per group (two per sex) and considers that an appropriate statistical analysis of the data to draw relevant conclusions is not possible in this study.

The GMO Panel considers that a subchronic feeding study in rodents on soybean 305423 × 40-3-2 is not needed on the basis of the molecular characterisation and comparative assessment.

3.5.3.2. 42-day feeding study in chickens for fattening

A 42-day feeding study with a total of 720 (half male and half female) chickens for fattening (1-day-old Ross x Cobb) was provided.[30] The birds were randomly allocated to six dietary treatment groups with 120 chicks per treatment (12 pens per treatment, six pens for males and six for females, 10 birds per pen). Birds were fed diets containing soybean 305423 × 40-3-2 (confirmed by real-time PCR, treated and not treated with the intended herbicides), and compared with those fed diets containing a negative segregant (generation BC_1F_7[31]) or three non-GM commercial varieties (93B86, 93B15 and 93M40). Diets were prepared containing equal quantities of defatted toasted soybean meal (26.5% in the starter phase diet, 23% in the grower phase diet, 21.5% in the finisher phase diet), soybean hull (1% in all phase diets) and soybean oil (0.5% in all phase diets), together with maize and other minor constituents. Before feed formulation, all soybean varieties were analysed for proximates, fibre fractions, minerals, amino acids, fatty acids and mycotoxins. The GM-HRA and CP4 EPSPS proteins were not detected in the toasted meal or hull.[32] The diets were isonitrogenous and

[30] Dossier: Part I – Annex 7.
[31] Additional information: 4/12/2013.
[32] Lower limit of quantification: 0.27 ng/mg.

isocaloric (confirmed by analysis). The starter phase diets (about 22% crude protein (CP), 3,124 kcal metabolisable energy (ME)/kg) were given until day 21, grower phase diets (about 20% CP, 3,151 kcal ME/kg) from day 22 to day 35, and finisher phase diets (about 18% CP, 3,175 kcal ME/kg) from day 36 until the end. Feed in mash form and water were provided to the birds for *ad libitum* intake.

Chickens were observed three times daily for clinical signs; deaths were recorded and necropsy performed on all birds found dead. Body weight and feed intake were measured every 7 days. At the end four birds per pen were taken for carcass evaluation (yield, dressing percentage, weight of thighs, breast, wings, legs, abdominal fat, kidney and whole liver). Performance and carcass trait tolerance intervals were constructed using data from the three groups of animals fed diets containing the non-GM commercial varieties. Differences between the control and 305423 × 40-3-2 groups (treated or not treated with the intended herbicides) were evaluated to determine if observed values were contained within the respective interval. The individual bird (not the pen) was considered as the experimental unit.

Overall mortality was low (< 2%) with no relevant difference between the groups. Overall no significant difference was seen in final body weight (about 1.9 kg) or feed:gain ratio (about 1.86) between the soybean 305423 × 40-3-2 and the negative segregant group. No significant differences were observed in carcass yield. All performance and carcass data fell within the confidence intervals established by the reference groups.

In recognition that a negative segregant is of limited value when used as control, the applicant provided another study[31] with the same design, in which the non-GM comparator Jack was compared to another GM soybean and to the same three commercial varieties used in the first study. No significant differences were observed for mortality (< 2%), performance parameters (final body weight ca. 1.9 kg, feed:gain ratio ca. 1.87), or carcass yield between Jack and the three commercial varieties. The applicant concluded that since both the GM soybean 305423 × 40-3-2 and its comparator Jack, albeit tested at different times, did not differ significantly from the three commercial varieties, no difference would be expected between the GM soybean 305423 × 40-3-2 and Jack.

The GMO Panel considers that a feeding study in chickens for fattening, in which material derived from a negative segregant is administered as the sole control material, has limitations, principally because of an inability to detect unintended effects. However, the GMO Panel accepts that the two studies taken in conjunction provide evidence that the defatted toasted GM soybean meal 305423 × 40-3-2 is as nutritious as non-GM soybean varieties.

3.5.4. Allergenicity

For allergenicity assessment, a weight-of-evidence approach is followed, taking into account all of the information on the newly expressed proteins, since no single piece of information or experimental method yields sufficient evidence to predict allergenicity (EFSA, 2006; Codex Alimentarius, 2009). In addition, when known functional aspects of the newly expressed protein or structural similarity to known adjuvants may indicate an adjuvant activity, the possible role of these proteins as adjuvants is considered. When newly expressed proteins with a potential adjuvant activity are expressed together, possible interactions increasing adjuvanticity and impacting the allergenicity of the GM crop are assessed.

3.5.4.1. Assessment of allergenicity of the newly expressed proteins[33]

For allergenicity, the GMO Panel has previously evaluated the safety of the GM-HRA and CP4 EPSPS proteins, and no concerns on allergenicity were identified in the context of the applications assessed (Table 1). No new information on allergenicity of the newly expressed GM-HRA and CP4 EPSPS proteins that might change the previous conclusions of the GMO Panel has become available. Based on current knowledge, and as none of the newly expressed proteins showed allergenicity, no reasons for concern regarding the simultaneous presence of these newly expressed proteins in this stack soybean affecting allergenicity were identified.

For adjuvanticity, no information available on the structure or function of the newly expressed GM-HRA and CP4 EPSPS proteins would suggest an adjuvant effect of the individual proteins or their simultaneous presence in soybean 305423 × 40-3-2 resulting in or increasing an eventual immunoglobulin E (IgE) response to a bystander protein.

[33] Dossier: Part I – Section D7.9.1; additional information: 16/4/2015.

3.5.4.2. Assessment of allergenicity of the whole GM plant[34]

Soybean is considered to be a common allergenic food[35] (OECD, 2012). Therefore, any potential change in the endogenous allergenicity of the GM plant when compared with that of its comparator(s) should be assessed (EFSA, 2006). Such assessments were performed for the single-event soybeans 305423 and 40-3-2, and no reasons for concern were identified by the GMO Panel (Table 1).

At the request of the GMO Panel, the applicant provided an assessment of the endogenous allergenicity of soybean 305423 × 40-3-2.[36] Specifically, the applicant performed two-dimensional electrophoresis of protein extracts of soybean 305423 × 40-3-2, its non-GM comparator and two non-GM commercial soybean reference varieties followed by western blotting using individual sera from 10 humans allergic/sensitised to soybean. This study showed no meaningful differences in the IgE-binding patterns between the extracts of proteins derived from soybean 305423 × 40-3-2, the non-GM comparator Jack and non-GM reference varieties. In addition, ELISA studies were also carried out using individual sera from 11 humans allergic/sensitised to soybean which confirmed the outcome of the previous study.

The GMO Panel considers that there is no evidence that the genetic modification might significantly change the overall allergenicity of soybean 305423 × 40-3-2 when compared with that of its non-GM comparator and non-GM commercial soybean reference varieties.

3.5.5. Nutritional assessment of GM food/feed

3.5.5.1. Human nutritional assessment

The main product for human consumption from soybean is the oil. The GMO Panel already assessed the nutritional consequences of the fatty acid profile modifications related to 305423 (EFSA GMO Panel, 2013). The fatty acid profile of soybean seeds 305423 × 40-3-2 is similar to that of soybean seeds 305423 (Section 3.4.3 and Table 6).[37] The fatty acid profile of the RDB oil of soybean 305423 × 40-3-2 is essentially the same as that of the unprocessed seeds (Table 8). Consequently, the GMO Panel concludes that the basis for the nutritional assessment made for soybean 305423 can also be used for soybean 305423 × 40-3-2.

The assessment of dietary exposure[38] embraced all possible uses of soybean 305423 × 40-3-2 oil, including both commercial and domestic uses of the oils.[39] Consumption data were taken from the UK National Diet and Nutrition Survey 2008-2010 (Bates et al., 2011). The subpopulations considered were toddlers (1–3 years), children (4–10 years), teenagers (11–18 years), adults (19–64 years) and elderly (≥ 65 years). The estimated dietary intakes (expressed as percentage of energy (E %) of the total diet) of fatty acid groups (saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), *cis*-n-3 PUFA and *cis*-n-6 PUFA (polyunsaturated fatty acids), trans fatty acids (TFA)) were based on the fatty acid composition of the unprocessed seeds of herbicide-treated soybean 305423 × 40-3-2 estimated in the first statistical comparative analysis of the 2011 field trials (Section 3.4.1), using three substitution levels (100%, 50% and 25%) of vegetable oils[40] with soybean 305423 × 40-3-2 oil. The GMO Panel selected the 100 % substitution as the most conservative scenario arising from both domestic and commercial use of the vegetable oils.

Calculations based on the full replacement scenario projected that fatty acid intakes would be increased for MUFA and *cis*-n-3 PUFA, reduced for *cis*-n-6 PUFA, slightly reduced for SFA, and unchanged for TFA (Table 9).

Similar changes were previously seen for soybean 305423 oil (EFSA GMO Panel, 2013). As in that case, the assessment of the nutritional impact of soybean 305423 × 40-3-2 oil is that these projected changes are generally small and would not impact on health and nutrition for average and high consumers of vegetable oils.

[34] Dossier: Part I – Section D7.9.2; additional information: 3/7/2014 and 14/4/2015.
[35] Directive 2007/68/EC of the European Parliament and of the Council of 27 November 2007 amending Annex IIIa to Directive 2000/13/EC of the European Parliament and of the Council as regards certain food ingredients. OJ L 310, 27.11.2007, p. 11–14.
[36] Additional information: 3/7/2014 and 16/4/2015.
[37] Additional information: 22/4/2014, 1/2/2016 and 19/5/2016.
[38] Spontaneous additional information: 30/7/2015.
[39] Food items considered are the targeted foods (fried fish, meat, potatoes, vegetables and other fried foods, savoury snacks and crackers) and other foods (salad dressings, margarines and spread, mayonnaise and home use of vegetable oils).
[40] Conventional soybean, rapeseed and sunflower oils. These three oils account for about 80% of vegetable oils available for consumption in the UK.

Dietary intake data for low consumers of vegetable oils (not available at the time of assessment of soybean 305423 oil) were provided in the context of this application. Thus, it was possible to assess dietary intakes and nutritional impacts for consumers of vegetable oils also at the low (5th) centile of vegetable oil intake when all vegetable oil is replaced by 305423 × 40-3-2 oil (full replacement scenario, Table 9). In the full replacement scenario, the estimated dietary intake for consumers of vegetable oils at the low (5th) centile of vegetable oil intake indicated that although a decrease in the intake of n-6 PUFA occurs in both male and females in all age groups, the resultant intakes would still exceed 1 E% (Table 9), which is the level below which clinical symptoms of linoleic deficiency have been observed (EFSA NDA Panel, 2010).

Table 9: Estimated daily intake (E%) of fatty acid groups before (B) and after (A) the replacement

Fatty acid group	Males						Females					
	5th %		50th %		95th %		5th %		50th %		95th %	
	B	A	B	A	B	A	B	A	B	A	B	A
Toddlers (1–3 years)												
SFA	8.9	8.9	14.7	14.4	20.6	20.1	10.2	9.4	14.8	14.5	19.7	19.4
MUFA	7.8	8.5	11.3	14.6	14.5	21.9	7.4	9.3	11.4	15.2	14.8	22.3
n-3 PUFA	0.4	0.5	0.7	1.3	1.1	3.3	0.4	0.5	0.7	1.5	1.3	4.0
n-6 PUFA	2.3	1.8	3.9	2.9	6.8	4.3	2.4	1.8	4.1	2.8	6.0	4.3
TFA	0.3	0.3	0.7	0.7	1.3	1.3	0.5	0.4	0.7	0.7	1.0	1.0
Children (4–10 years)												
SFA	9.3	9.1	13.4	13.1	18.0	18.0	9.1	8.8	13.3	13.0	17.7	17.6
MUFA	8.6	10.1	11.9	16.7	15.2	24.7	8.7	10.2	12.2	17.2	15.9	27.0
n-3 PUFA	0.5	0.6	0.8	1.6	1.3	3.2	0.5	0.6	0.9	1.7	1.4	3.8
n-6 PUFA	2.7	2.1	4.4	3.2	6.6	4.4	2.8	2.2	4.6	3.3	6.7	5.0
TFA	0.4	0.4	0.7	0.7	1.0	1.0	0.4	0.4	0.7	0.7	1.2	1.2
Teenagers (11–18 years)												
SFA	8.4	8.3	12.5	12.3	16.9	16.8	7.7	7.5	12.4	12.2	16.5	16.2
MUFA	8.8	11.1	12.5	17.7	16.2	26.3	8.3	10.2	12.9	19.4	16.8	31.8
n-3 PUFA	0.5	0.7	0.9	1.9	1.5	5.9	0.5	0.7	1.0	2.1	1.7	5.1
n-6 PUFA	3.2	2.2	4.8	3.4	6.9	5.0	2.8	2.1	4.9	3.4	7.3	5.3
TFA	0.4	0.4	0.7	0.7	1.1	1.1	0.4	0.4	0.8	0.7	1.1	1.1
Adults (19–64 years)												
SFA	6.4	6.4	12.1	11.8	18.4	17.9	6.8	6.9	12.1	11.8	17.5	17.2
MUFA	6.8	8.4	11.9	16.1	16.5	24.0	6.4	8.1	11.6	15.7	16.1	24.0
n-3 PUFA	0.5	0.5	1.0	1.4	1.8	3.4	0.5	0.6	1.0	1.6	1.9	3.3
n-6 PUFA	2.6	2.0	4.8	3.4	7.3	5.2	2.7	1.9	4.9	3.5	7.8	5.9
TFA	0.3	0.3	0.7	0.7	1.2	1.2	0.3	0.3	0.7	0.7	1.2	1.2
Elderly (≥ 65 years)												
SFA	8.3	8.0	13.8	13.5	19.6	19.3	7.4	7.1	14.0	13.8	20.2	20.7
MUFA	8.6	9.2	11.9	15.3	15.6	22.9	8.3	9.1	11.7	14.7	15.9	22.7
n-3 PUFA	0.6	0.6	1.1	1.4	2.0	3.0	0.5	0.6	1.1	1.3	2.1	2.4
n-6 PUFA	2.4	2.2	4.8	3.4	7.3	4.8	2.7	2.2	4.8	3.5	7.5	5.6
TFA	0.4	0.4	0.9	0.8	1.4	1.4	0.4	0.4	0.8	0.8	1.3	1.3

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; TFA: trans fatty acids. Predicted changes in the total diet with respect to fatty acid groups (SFA, MUFA, PUFA, TFA) through replacement of all consumed vegetable oils (rapeseed, sunflower, blended vegetable oils) by soybean 305423 × 40-3-2 oil are given as percentage of total energy in different age groups for the 5th, 50th and 95th centile consumers.

The GMO Panel noted that in the final statistical comparative analysis of the 2011 field trials (Section 3.4.1, 3.4.3)[41] some of the estimated mean values of the fatty acid levels for soybean 305423 × 40-3-2 seeds changed with respect to the estimates used for the exposure assessment. The GMO

[41] Additional information: 19/5/2016.

Panel concluded that the relative proportions of SFA, MUFA, n-3 and n-6 PUFA, and of TFA obtained in the two different statistical analyses are essentially identical. Considering that former analyses have demonstrated that the fatty acid pattern of the soybean seed is reflected in the RBD oil derived from the seed, the GMO Panel concludes that a new exposure assessment with repeated substitution scenarios is not needed. The GMO Panel also notes that the estimated content of linoleic acid and (9,15) isomer of linoleic acid are higher in the final analysis compared to the first analysis (from 3.76% FA to 4.17% FA and from 0.645% FA to 0.752% FA, respectively). Therefore, 305423 × 40-3-2 soybean seed is even less likely to induce linoleic acid deficiency in low consumers of vegetable oils with complete substitution by 305423 × 40-3-2 soybean oil.

In conclusion, the profile of fatty acid intake, after substituting soybean 305423 × 40-3-2 oil for conventional vegetable oils, should not have adverse impact on consumer's health and nutrition regarding four fatty acids groups (SFA, MUFA, n-3 PUFA and TFA). Changes in n-6 PUFA might give rise to concern, given the proximity of the intake values for low consumers to the level below which deficiency of linoleic acid (the main dietary fatty acid of the n-6 PUFA group) might occur. However, considering the conservative nature of the full replacement scenario in the consumer group with the lowest consumption of vegetable oils in the exposure assessment, the size of the observed differences would not be expected to introduce adverse effects on human health with respect to n-6 PUFA intake.

Other soybean products for human consumption (for example, lecithin[42]) are not expected to differ in their composition, except for their fatty acid 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.

3.5.5.2. Animal nutritional assessment

Defatted toasted soybean meal represents the most common soybean by-product used in animal feed formulations, with around 90% of the defatted toasted soybean meal entering the feed chain in the EU mainly to poultry, pig and cattle. 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.[43]

Compositional data indicates that the defatted toasted soybean meal from soybean 305423 × 40-3-2 is expected to deliver the same nutrition as its non-GM comparator and other non-GM commercial varieties. This is consistent with the assessment of the single event 305423 (EFSA GMO Panel, 2013) and was confirmed by the results of a feeding study in chickens for fattening (Section 3.5.3).

3.5.6. Conclusion

The safety assessment identified no concerns regarding the potential toxicity and allergenicity of the proteins GM-HRA and CP4 EPSPS, newly expressed in soybean 305423 × 40-3-2, and found no evidence that the genetic modification might significantly change the overall allergenicity of soybean 305423 × 40-3-2. Nutritional assessment of soybean 305423 × 40-3-2 oil and oil-containing food products did not identify concerns for human health and nutrition. The contribution of fatty acids from soybean 305423 × 40-3-2 in other soybean products to overall human exposure would be small and is not expected to affect the conclusion on human health and nutrition. Based on the assessment of the single event 305423, on compositional data for soybean 305423 × 40-3-2, and on the results of a feeding study in chickens for fattening, the GMO Panel concludes that feedingstuffs derived from defatted toasted 305423 × 40-3-2 soybean meal are safe and as nutritious as those derived from other non-GM soybean varieties.

3.6. Environmental risk assessment

3.6.1. Evaluation of relevant scientific data

Considering the scope of application EFSA-GMO-NL-2007-47 (which excludes cultivation), the environmental risk assessment (ERA) of soybean 305423 × 40-3-2 is mainly concerned with: (1) the exposure of bacteria to recombinant DNA in the gastrointestinal tract of animals fed GM material and bacteria present in environments exposed to their faecal material (manure and faeces); and (2) the

[42] Additional information: 24/7/2014.
[43] Deutscher Verband für Tiernahrung, personal communication, 29/7/2011.

accidental release into the environment of viable soybean 305423 × 40-3-2 seeds during transportation and processing (EFSA GMO Panel, 2010a).

3.6.2. Persistence and invasiveness of the GM plant[44]

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

The applicant presented agronomic and phenotypic data on soybean 305423 × 40-3-2 gathered from field trials conducted in soybean growing areas in North America (Section 3.4.1). The data showed differences with the non-GM comparator and the three additional comparators (the negative segregant and the two parental lines, soybean 305423 and soybean 40-3-2) for several endpoints and equivalence with the non-GM reference varieties for all the endpoints. Due to the low survival capacity of soybean, the observed differences are unlikely to change the fitness (e.g. survival, fecundity, competitiveness) or invasiveness characteristics of soybean 305423 × 40-3-2 plants.

It is considered very unlikely that soybean 305423 × 40-3-2 will differ from conventional soybean varieties in its ability to survive until subsequent seasons, or to establish occasional feral plants under European environmental conditions in case of accidental release into the environment of viable soybean 305423 × 40-3-2 seeds during transportation and processing.

The expected change in seed fatty acid composition in soybean 305423 × 40-3-2 resulting from the newly inserted *gm-fad2-1* gene (encoding the Δ6 desaturase protein from *G. max*) is not known to provide a potential agronomic advantage. The *gm-hra* and CP4 *epsps* genes coding for herbicide tolerance traits can provide a potential selective advantage for this GM soybean plant when glyphosate-based and ALS-inhibiting herbicides are applied. However, in case of accidental release intro the environment of viable soybean 305423 × 40-3-2 seeds during transportation and processing, establishment and survival of this GM soybean in the EU is limited by the biotic and abiotic factors described above.

The GMO Panel is not aware of any scientific report of increased survival capacity, including overwintering, of existing GM soybeans varieties (Dorokhov et al., 2004; Owen, 2005; Bagavathiannan and Van Acker, 2008; Lee et al., 2009). Therefore, the GMO Panel is of the opinion that the likelihood of environmental effects of soybean 305423 × 40-3-2 in Europe will not be different from that of conventional soybean varieties.

3.6.3. Effects of gene transfer[45]

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

1) Plant-to-microorganism gene transfer[5]

The potential for horizontal gene transfer of the recombinant DNA of the single events has already been assessed in previous opinions (see Table 1) and no concern for an unlikely, but theoretically possible, horizontal gene transfer of the recombinant genes to bacteria in the gut or other receiving environments was identified.

Synergistic effects of the recombinant genes, for instance due to combinations of recombinogenic sequences, which would cause an increase in the likelihood for horizontal gene transfer or a selective advantage, were not identified.

[44] Dossier: Part I – Section D9.1; additional information: 1/2/2016 and 19/5/2016.
[45] Dossier: Part I – Section D9.3.

Therefore, in line with its previous assessments of soybean events 305423 and 40-3-2, the GMO Panel concludes that the horizontal gene transfer from soybean 305423 × 40-3-2 to bacteria is highly unlikely, theoretically possible but does not raise a safety concern.

2) Plant-to-plant gene transfer

Considering the scope of application EFSA-GMO-NL-2007-47 and the biology of soybean, a possible pathway to harm is the potential of occasional feral GM soybean plants originating from seed import spills to transfer recombinant DNA to sexually cross-compatible plants.

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

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

However, cross-pollination rates as high as 6.3% have been reported for closely spaced plants (Ray et al., 2003), suggesting the potential for some within-crop gene flow in soybean. These results indicate that natural cross-pollination rates can fluctuate significantly among different soybean varieties under particular environmental conditions such as a favourable climate for pollination and an abundance of pollinators (Gumisiriza and Rubaihayo, 1978; Kikuchi et al., 1993; Ahrent and Caviness, 1994; Ray et al., 2003; Lu, 2005).

For plant-to-plant gene transfer to occur, imported soybean 305423 × 40-3-2 seeds need to be processed outside the importing ports, transported into regions of soybean production in Europe, spilled during transportation, germinate and develop into plants in the very close vicinity of soybean fields, and there needs to be an overlap of flowering periods and environmental conditions favouring cross-pollination. It must be noted that most soybean 305423 × 40-3-2 seeds are processed in the countries of production or in ports of importation. The overall likelihood of cross-pollination between occasional feral GM soybean plants and cultivated soybean is therefore extremely low.

In conclusion, even if cross-pollination would occur, the GMO Panel is of the opinion that the likelihood of environmental effects as a consequence of spread of genes from occasional feral soybean plants in Europe will not differ from that of conventional soybean varieties (see Section 3.6.2).

3.6.4. Interactions of the GM plant with target organisms[46]

Considering the scope of application EFSA-GMO-NL-2007-47, and the absence of target pests, potential interactions of the GM plant with target organisms are not considered a relevant issue by the GMO Panel.

3.6.5. Interactions of the GM plant with non-target organisms[47]

Considering the scope of application EFSA-GMO-NL-2007-47, and the low level of exposure to the environment, potential interactions of spilled seeds or occasional feral soybean 305423 × 40-3-2 arising from seed import spills with non-target organisms are not considered a relevant issue by the GMO Panel.

[46] Dossier: Part I – Section D9.4.
[47] Dossier: Part I – Section D9.5.

3.6.6. Interactions with the abiotic environment and biogeochemical cycles[48]

Considering the scope of application EFSA-GMO-NL-2007-47, and the low level of exposure to the environment, potential interactions of spilled seeds or occasional feral soybean 305423 × 40-3-2 arising from seed import spills with the abiotic environment and biogeochemical cycles are not considered a relevant issue by the GMO Panel.

3.6.7. Conclusion

In the case of accidental release into the environment of viable seeds of soybean 305423 × 40-3-2, there are no indications of an increased likelihood of the establishment and spread of occasional feral soybean 305423 × 40-3-2 plants, unless these plants are exposed to ALS-inhibiting or glyphosate-based herbicides

Considering the scope of the application EFSA-GMO-NL-2007-47, interactions of soybean 305423 × 40-3-2 with the biotic and abiotic environment are not considered to be relevant issues. The unlikely but theoretically possible transfer of the recombinant genes from soybean 305423 × 40-3-2 to bacteria does not raise a safety concern for these bacteria owing to the lack of a selective advantage.

Therefore, considering the introduced traits, the outcome of the comparative analysis, the routes of exposure and the limited exposure levels, the GMO Panel concludes that soybean 305423 × 40-3-2 would not raise safety concerns in the event of accidental release of viable GM soybean seeds into the environment.

3.7. Post-market monitoring

3.7.1. Post-market monitoring of GM food/feed

Considering the intended, altered nutritional composition of soybean 305423 × 40-3-2, a proposal for a post-market monitoring (PMM) plan needs to be provided by the applicant (EFSA GMO Panel, 2011a).

For specific labelling, the applicant proposed that, for example, operators handling products containing or consisting of oil produced from soybean 305423 × 40-3-2 shall be required to label these products with the words 'genetically modified soybean with altered fatty acid profile'.[49] The GMO Panel considers that this proposal is consistent with the compositional data provided for this soybean.

3.7.2. Post-market environmental monitoring[50]

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

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

The PMEM plan proposed by the applicant includes: (1) the description of a monitoring approach involving operators (federations involved in soybean import and processing), reporting to the applicants, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment; (2) a coordinating system established by EuropaBio for the collection of information recorded by the various operators (Lecoq et al., 2007; Windels et al., 2008); and (3) the 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 period. The GMO Panel considers that the scope of the post-market environmental monitoring plan provided by the applicant is consistent with the scope of soybean 305423 × 40-3-2. As the ERA does not cover cultivation and did not identify potential adverse environmental effects from soybean 305423 × 40-3-2, no case-specific monitoring is necessary. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

[48] Dossier: Part I – Section D9.8 and D10.
[49] Additional information: 3/9/2015.
[50] Dossier: Part I – Section D11; additional information: 26/2/2014.

3.7.3. Conclusion

The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of soybean 305423 × 40-3-2 and the GMO Panel guidelines on the PMEM of GM plants (EFSA GMO Panel, 2011b).

4. Overall conclusions

No new data on the single soybean events 305423 and 40-3-2 that would lead to a modification of the original conclusions on their safety were identified.

Based on the molecular, agronomic, phenotypic and compositional characteristics, the GMO Panel considers that the combination of soybean single events 305423 and 40-3-2 in the two-event stack soybean 305423 × 40-3-2 did not raise concerns regarding food and feed safety or nutrition. The combination of the newly expressed proteins in the two-event stack soybean does not raise concerns for human and animal health.

The agronomic and phenotypic characteristics and the composition of soybean 305423 × 40-3-2 were compared under field conditions with those of the non-GM comparator Jack and three additional comparators (a negative segregant of soybean 305423 × 40-3-2 and the two single-event parental lines soybean 305423 and 40-3-2), and tested for equivalence with a set of non-GM soybean reference varieties. Soybean 305423 × 40-3-2 differs from the non-GM comparator Jack and the negative segregant, and is not equivalent to the non-GM soybean reference varieties, in having an altered fatty acid profile. The changes in fatty acid profile are consistent with those observed in the single-event soybean 305423, including the increase in oleic acid content (the intended trait). No further assessment for food and feed safety was needed for the other differences, or any other lack of equivalence, identified in the agronomic and phenotypic characteristics and composition of soybean 305423 × 40-3-2. The comparison with the two parental lines (the single events soybean 305423 and 40-3-2) did not reveal any potential interaction that could be of concern for food and feed safety.

Nutritional assessment on soybean 305423 × 40-3-2 oil and oil-containing food products did not identify concerns for human health and nutrition. The contribution to overall human exposure of fatty acids from other soybean 305423 × 40-3-2 products would be small and is not expected to affect the conclusion on human health and nutrition. Based on the assessment of the single event 305423, on compositional data for soybean 305423 × 40-3-2, and on the results of a feeding study in chickens for fattening, the GMO Panel concludes that there are no concerns regarding the use of feedingstuffs derived from defatted toasted 305423 × 40-3-2 soybean meal.

Considering the intended altered soybean 305423 × 40-3-2 nutritional composition, a proposal for a PMM plan needs to be provided by the applicant (EFSA GMO Panel, 2011a).

In the case of accidental release into the environment of viable seeds of soybean 305423 × 40-3-2, there are no indications of an increased likelihood of establishment and spread of occasional feral soybean 305423 × 40-3-2 plants, unless these plants are exposed to ALS-inhibiting or glyphosate-based herbicides. The unlikely but theoretically possible transfer of the recombinant genes from soybean 305423 × 40-3-2 to bacteria does not raise a safety concern for these bacteria owing to the lack of a selective advantage. Potential interactions of soybean 305423 × 40-3-2 with the biotic and abiotic environment were not considered a relevant issue by the GMO Panel. Therefore, considering the introduced traits, the outcome of the comparative analysis, the routes of exposure and the limited exposure levels, the GMO Panel concludes that soybean 305423 × 40-3-2 would not raise safety concerns in the event of accidental release of viable GM soybean seeds into the environment. The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of soybean 305423 × 40-3-2 and the GMO Panel guidelines on the PMEM of GM plants.

In conclusion, the GMO Panel considers that the information available for soybean 305423 × 40-3-2 addresses the scientific comments raised by the Member States and that soybean 305423 × 40-3-2, as described in this application, is as safe as the non-GM comparator and non-GM conventional soybean varieties with respect to potential effects on human and animal health and the environment in the context of its scope.

Considering the modified composition and nutritional values of soybean 305423 × 40-3-2, the GMO Panel agrees with the specific labelling proposal provided by the applicant, in accordance with Articles 13(2)(a) and 25(2)(c) of Regulation (EC) No 1829/2003.

Documentation requested and provided to EFSA

1) Letter from the Competent Authority of the Netherlands received 24 September 2007 concerning a request for authorisation for the placing on the market of genetically modified soybean 305423 × 40-3-2 for food and feed uses, import and processing submitted in accordance with Regulation (EC) No 1829/2003 by Pioneer Overseas Corporation.
2) Acknowledgement letter dated 25 September 2007 from EFSA to the Competent Authority of the Netherlands.
3) Letter from EFSA to applicant dated 12 November 2007 requesting additional information under completeness check.
4) Letter from applicant to EFSA received on 20 November 2007 providing additional information under completeness check.
5) Letter from EFSA to applicant dated 19 February 2008 delivering the 'Statement of Validity' for application EFSA-GMO-NL-2007-47 (soybean 305423 × 40-3-2) submitted by Pioneer Overseas Corporation under Regulation (EC) No 1829/2003.
6) Letter from EFSA to applicant dated 29 February 2008 stopping the clock due the on-going assessment of the single event 305423 (application reference EFSA-GMO-UK-2007-45).
7) Letter from EFSA to applicant dated 8 December 2009 requesting additional information and maintaining the clock stopped.
8) Letter from applicant to EFSA received on 27 January 2010 providing additional information.
9) Letter from EFSA to applicant dated 15 December 2010 maintaining the clock stopped due to the on-going assessment of the single event 305423 (application reference EFSA-GMO-UK-2007-45).
10) Letter from applicant to EFSA received on 5 December 2013 providing additional information spontaneously.
11) Letter from EFSA to applicant dated 10 December 2013 re-starting the clock due to the finalisation of the assessment of the single event 305423 (application reference EFSA-GMO-UK-2007-45).
12) Letter from EFSA to applicant dated 13 February 2014 requesting additional information and stopping the clock.
13) Letter from applicant to EFSA received on 26 February 2014 providing additional information.
14) Letter from EFSA to applicant dated 1 April 2014 requesting additional information and maintaining the clock stopped.
15) Letter from applicant to EFSA received on 22 April 2014 providing additional information.
16) Letter from EFSA to applicant dated 22 May 2014 requesting additional information and maintaining the clock stopped.
17) Letter from applicant to EFSA received on 20 June 2014 providing additional information.
18) Letter from applicant to EFSA received on 3 July 2014 providing additional information.
19) Letter from EFSA to applicant dated 4 July 2014 requesting additional information and maintaining the clock stopped.
20) Letter from applicant to EFSA received on 24 July 2014 providing additional information.
21) Letter from EFSA to applicant dated 25 July 2014 requesting additional information and maintaining the clock stopped.
22) Letter from EFSA to applicant dated 4 September 2014 requesting additional information and maintaining the clock stopped.
23) Letter from applicant to EFSA received on 9 September 2014 providing additional information.
24) Letter from applicant to EFSA received on 6 October 2014 providing additional information.
25) Letter from EFSA to applicant dated 10 November 2014 requesting additional information and maintaining the clock stopped.
26) Letter from applicant to EFSA received on 1 December 2014 providing additional information.
27) Letter from EFSA to applicant dated 10 February 2015 requesting additional information and maintaining the clock stopped.
28) Letter from applicant to EFSA received on 16 April 2015 providing additional information and complementing additional information already received spontaneously.

29) Letter from EFSA to applicant dated 21 April 2015 requesting additional information and maintaining the clock stopped.
30) Letter from EFSA to applicant dated 5 May 2015 requesting additional information and maintaining the clock stopped.
31) Letter from applicant to EFSA received on 13 May 2015 providing additional information.
32) Letter from applicant to EFSA received on 29 June 2015 providing additional information.
33) Letter from applicant to EFSA received on 30 July 2015 providing additional information spontaneously.
34) Letter from applicant to EFSA received on 3 September 2015 providing clarifications on the Labelling proposal of the application.
35) Letter from EFSA to applicant dated 10 September 2015 requesting additional information and maintaining the clock stopped.
36) Letter from applicant to EFSA received on 25 September 2015 providing additional information.
37) Letter from EFSA to applicant dated 20 October 2015 re-starting the clock (re-start date applicable was 14 October 2015).
38) Letter from applicant to EFSA received on 9 November 2015 providing additional information spontaneously.
39) Letter from EFSA to applicant dated 8 December 2015 requesting additional information and stopping the clock.
40) Letter from applicant to EFSA received on 1 February 2016 providing additional information.
41) Letter from EFSA to applicant dated 21 April 2016 requesting additional information and maintaining the clock stopped.
42) Letter from applicant to EFSA received on 19 May 2016 providing additional information.
43) Email from EFSA to applicant dated 20 May 2016 re-starting the clock (re-start date applicable was 19 May 2016).

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Abbreviations

ADF	acid detergent fibre
AHAS	acetohydroxyacid synthase
ALS	acetolactate synthase
CaMV	cauliflower mosaic virus
CHT	conventional herbicide treatment
CTP	chloroplast transit peptide
Dw	dry weight
GMO Panel	Panel on Genetically Modified Organisms of the European Food Safety Authority
ELISA	enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	environmental risk assessment
FA	fatty acid
FAD	Fatty Acid Desaturase
GM	genetically modified
GMO	genetically modified organism
GM-HRA	*Glycine max* herbicide-resistant ALS
IgE	immunoglobulin E
IHT	intended herbicide treatment
KTi3	Kunitz trypsin inhibitor gene 3
MUFA	monounsaturated fatty acids
NOS	nopaline synthase
NDF	neutral detergent fibre
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PMEM	post-market environmental monitoring
PMM	post-market monitoring
PUFA	polyunsaturated fatty acids
RBD	refined bleached deodorised
SAMS	*S*-adenosyl-L-methionine synthetase
SFA	saturated fatty acids
TFA	trans fatty acids
TIU	trypsin inhibitor units
UTR	untranslated region