STATEMENT

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Risk assessment of new sequencing information on genetically modified soybean event 305423

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Abstract

The GMO Panel has previously assessed genetically modified (GM) soybean 305423 as a single event and as part of a two-event stack, 305423 × 40-3-2. These soybean events were found to be as safe as their conventional counterparts and other appropriate comparators with respect to potential effects on human and animal health and the environment. On 23 February 2017, European Commission requested EFSA to analyse new nucleic acid sequencing data and updated bioinformatics data for soybean event 305423 and to indicate whether the previous conclusions of the GMO Panel on the previously assessed GM soybeans remain valid. The new sequencing data indicated a four base pair (bp) difference compared to the sequencing data originally provided: one bp located in the genomic 3′ flanking region, two bp located in a gene silencing cassette and one bp in a partial promoter. These bp reported as differences in the new nucleic acid sequencing data on soybean event 305423 were already present in the original plant material used for the risk assessment. Thus, with the exception of bioinformatics analyses, including an off-target search with the dsRNA expression cassette, the studies performed for the risk assessment of the single event soybean 305423 and the two-event stack soybean 305423 × 40-3-2 remain valid. The new sequencing data and the bioinformatic analyses performed on the new sequence including the RNAi off-target search, did not give rise to safety issues. Therefore, EFSA concludes that the original risk assessment of the single soybean event 305423 and the two-event stack soybean 305423 × 40-3-2 remains valid.

Keywords: GMO, soybean (*Glycine max*), 305423, nucleotide sequence, Regulation (EC) No 1829/2003

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1. Introduction

Genetically modified (GM) soybean event 305423 was developed through particle bombardment with plasmid fragments PHP19340A[1] and PHP17752A,[2] and contains the *gm-fad2-1* and *gm-hra* expression cassettes, leading to a modified fatty acid profile in the GM plant and tolerance to acetolactate synthase-inhibiting herbicides.

The GMO Panel has previously assessed soybean event 305423 as a single event and as part of a two-event soybean stack (see Table 1).

Table 1: EFSA GMO Panel scientific opinions on soybean event 305423

Event	Application	EFSA Scientific Opinions
305423	EFSA-GMO-NL-2007-45	EFSA GMO Panel (2013)
305423 × 40-3-2	EFSA-GMO-NL-2007-47	EFSA GMO Panel (2016)

1.1. Background and Terms of Reference as provided by the requestor

On 25 November 2016, Pioneer sent to the European Commission new sequencing information relating to soybean event 305423, on the basis of Articles 9 and 21 of Regulation (EC) 1829/2003. On 23 February 2017, the European Commission requested the European Food Safety Authority (EFSA) to evaluate the data and analyses provided by Pioneer and indicate whether, on the basis of these elements, the conclusions of adopted opinions for soybean 305423 as a single event or as part of stacked events have to be adapted. Subsequently, the EFSA has evaluated the data and methodology provided for soybean event 305423 and considered these elements in the context of previous conclusions.

2. Data and methodologies

2.1. Data

The applicant followed the relevant parts of the GMO Panel guidelines for the risk assessment of GM plants (EFSA GMO Panel, 2011) to investigate the insert sequence and to perform the bioinformatics analyses. In delivering this statement, EFSA took into account the appropriate principles described in the GMO Panel guidelines for the risk assessment of GM plants (EFSA GMO Panel, 2011) and Implementing Regulation (EU) No 503/2013.

2.2. Methodologies

In delivering this statement, EFSA took into account information provided by the applicant and relevant scientific publications.

2.2.1. Sequence information previously submitted to EFSA for soybean 305423

The applicant had previously submitted information on the sequence of soybean event 305423, as part of application EFSA-GMO-NL-2007-45.[3] Soybean event 305423 contains four genetically linked insertions as follows:

- Insert region 1 (12,928 base pair (bp)), which contains one intact PHP19340A fragment, one intact PHP17752A fragment, and three truncated PHP19340A fragments;
- Insert region 2 (2,331 bp), which contains one truncated PHP19340A fragment;
- Insert region 3 (2,063 bp), which contains one truncated PHP19340A fragment and a non-functional 495 bp fragment of the plasmid backbone;
- Insert region 4 (5,020 bp), which contains two truncated PHP19340A fragments in an inverted repeat configuration.

[1] Plasmid fragment PHP19340A contains the *gm-fad2-1* cassette, which consists of the Kunitz trypsin inhibitor gene 3 (KTi3) promoter, the *gm-fad2-1* gene fragment of the coding region of the microsomal omega-6 desaturase gene 1 from *Glycine max*, and the KTi3 terminator.

[2] Plasmid fragment PHP17752A contains the *gm-hra* cassette, which consists of S-adenosyl-L-methionine synthetase (SAMS) promoter, the modified *Glycine max* acetolactate synthase (*als*) gene (*gm-hra*) and the *als* terminator.

[3] Part I Technical dossier – Annex 2 (confidential information).

2.2.2. New information for soybean event 305423 submitted as part of the current mandate

The applicant has recently resequenced the soybean event 305423 in a stack and compared this sequence with the original soybean event sequence reported in 2007.[4] This revealed four bp differences which are: one bp located in the 3′ flanking region of insert region 2, two bp located in the *gm-fad2-1* gene of insert region 4, and one bp in KTi3 promoter of insert region 4 (see Table 2).

Table 2: Identified differences in the sequence of the inserts and flanking regions in soybean event 305423

Identified difference	Position*	Reported in 2007	Reported in 2016
3′ flanking region (region 2)	10583	GGA**G**GGA	GGA**T**GGA
gm-fad2-1 gene (region 4)	5615–5616	TTA**GA**ATA	TTA**AT**ATA
Partial KTi3 promoter (region 4)	7371	TTT**T**TTT	TTT**C**TTT

*: Positions are relative to each insertion region.

Genomic DNA from the same soybean event 305423 material analysed in 2007 was used as a template to amplify and sequence the four insertion regions.[5] The results indicated that the four bp differences found in the stack were present in the original soybean event 305423 material.

For the reported differences, the applicant evaluated the impact on the original bioinformatics analyses. The one bp difference identified in the 3′ flanking region of insert region 2 was found not to impact the outcome of the flanking border analysis or the analysis of the ORFs spanning the junctions between this insert region and soybean genomic DNA, therefore bioinformatics analyses were not performed for the updated sequence. For insert region 4, which contained the other three reported differences, the applicant carried out bioinformatic analyses using the updated nucleotide sequence in order to investigate (1) if any open reading frame (ORF) present within the insert or spanning the junctions between the insert and genomic DNA shows similarity to known allergens or toxins, and (2) if the insert contains sequences that would facilitate horizontal gene transfer (HGT) to microorganisms.

As two bp of the reported differences were in a *gm-fad2-1* dsRNA cassette in insert region 4, EFSA requested the applicant to perform a search for potential off-target genes with all putative siRNA sequences obtained from the *gm-fad2-1* dsRNA cassette present in soybean 305423. The applicant performed the search and documented all criteria used in the off-target prediction program. The results were processed and a detailed risk assessment was provided.[6]

3. Assessment

The provided data indicated that the sequence differences in soybean event 305423 were already present in the original material used in application EFSA-GMO-NL-2007-45 (EFSA GMO Panel, 2013).

Regarding the one bp sequence difference identified in region 2, which was located in the 3′ flanking region of the insert, and found to not impact any of the ORFs spanning the junction site, no further bioinformatic analysis was necessary.

Bioinformatic analyses performed with the updated sequence of insert region 4 (containing 3-bp sequence differences identified) with regard to potential similarity with allergens or toxins, as well as the implications of these differences on the potential for HGT, were considered relevant for the current assessment. The bioinformatic searches for similarity to allergens were performed according to EFSA guidelines (EFSA GMO Panel, 2010, 2011). Results indicate that none of the ORFs containing the three reported differences show similarity with known allergens or toxins. Sequence analysis did not identify any similarity between the region containing these differences and microbial sequences. Therefore, these sequence differences do not affect the likelihood of HGT.

The *gm-fad2-1* dsRNA cassette containing the two bp sequence difference was assessed for its potential impact on off-target gene expression regulation. The bioinformatics off-target search provided by the applicant was assessed based on the prediction program search criteria and the number of siRNAs predicted to bind to the respective off-target genes. The list of potential off-target genes was further assessed based on the predicted function and biological relevance of the off-target

[4] Annex 2 PHI-R070-Y16 (confidential information).
[5] Annex 1 PHI-2016-063 (confidential information).
[6] Additional information: 31 May 2017 (confidential information).

genes and the comparative assessment information described in the GMO Panel scientific opinions on soybean event 305423 (EFSA GMO Panel, 2013, 2016, Table 1). Having taken all this available data into account, EFSA concluded that the outcome of the off-target search does not impact the original risk assessment of soybean event 305423.

The other studies performed for the risk assessment of soybean event 305423 are not affected by the new sequencing information.

4. Conclusions

Based on analysis of the provided data, it can be concluded that the sequence of soybean event 305423 present in the original material used for the risk assessment process of the single event soybean 305423 and the two-event stack soybean 305423 × 40-3-2 event already contained the nucleotide differences reported in 2016. The bioinformatic analyses, including an RNAi off-target search performed on the corrected sequence, did not give rise to safety issues. Studies other than bioinformatics are not affected by this new sequence information. EFSA concludes that the original risk assessment of the single soybean event 305423 and the two-event stack soybean 305423 × 40-3-2 remains valid.

Documentation provided to EFSA

1) Letter from the European Commission, received on 23 February 2017, concerning a request to analyse new sequencing information for soybean event 305423.
2) Acknowledgement letter dated 23 March 2017 from EFSA to the European Commission.
3) Letter from EFSA to applicant dated 7 April 2017 requesting additional information.
4) Letter from applicant to EFSA received on 26 April 2017 providing additional information.
5) Letter from EFSA to applicant dated 11 May 2017 requesting additional information.
6) Letter from applicant to EFSA received on 30 May 2017 providing additional information.
7) Letter from EFSA to the European Commission requesting a deadline extension.

References

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

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

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EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2016. 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 9 40-3-2 for food and feed uses, import and processing under Regulation (EC) No 1829/2003. EFSA Journal 2016; 14(8):4566, 31 pp. https://doi.org/10.2903/j.efsa.2016.4566

Abbreviations

bp	base pair
dsRNA	double-stranded ribonucleic acid
GMO	genetically modified organism
HGT	horizontal gene transfer
ORF	open reading frame
RNAi	ribonucleic acid interference
siRNA	small interfering ribonucleic acid