

APPROVED: 19 July 2017

doi: 10.2903/j.efsa.2017.4968

Risk assessment of new sequencing information on genetically modified soybean event 40-3-2

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Abstract

The GMO Panel has previously assessed genetically modified (GM) soybean 40-3-2 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 4 April 2017, European Commission requested EFSA to analyse new nucleic acid sequencing and updated bioinformatics data for soybean event 40-3-2 and to indicate whether the conclusions of the GMO Panel on the previously assessed GM soybeans remain valid. The new sequencing data indicated that, the sequence of soybean event 40-3-2 as present in the stacked soybean 305423 × 40-3-2 contains an additional nucleotide in the 5' flanking region of a 72 bp additional insert of CP4 EPSPS present in soybean 40-3-2. Re-examination of the original sequencing data of the single soybean event 40-3-2 by the applicant, indicated that this additional nucleotide was already present in the original plant material used for the risk assessment of soybean event 40-3-2. Thus, with the exception of bioinformatics analyses, the studies performed for the risk assessment of the single event soybean 40-3-2 and the two-event stack soybean 305423 × 40-3-2 remain valid. The updated bioinformatic analyses performed on the corrected sequence did not give rise to safety issues. Therefore, EFSA concludes, based on the information provided, that the original risk assessment of the soybean event 40-3-2 as a single and the stacked soybean 305423 × 40-3-2 remains valid.

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Keywords: GMO, soybean (*Glycine max*), 40-3-2, nucleotide sequence, Regulation (EC) No 1829/2003

Requestor: European Commission

Question number: EFSA-Q-2017-00399

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Acknowledgements: EFSA wishes to thank the following for the support provided to this scientific output: Irina Olaru for the preparatory work on this scientific output.

Suggested citation: EFSA (European Food Safety Authority), Papadopoulou N, Ramon M and Waigmann E, 2017. Statement on the risk assessment of new sequencing information on genetically modified soybean event 40-3-2. *EFSA Journal* 2017;15(8):4968, 6 pp. <https://doi.org/10.2903/j.efsa.2017.4968>

ISSN: 1831-4732

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1. Introduction

Genetically modified (GM) soybean event 40-3-2 was developed through particle bombardment with plasmid PV-GMGT04, and expresses the 5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium* sp. strain CP4 (CP4-EPSPS), which confers tolerance to glyphosate. The 40-3-2 soybean contains two inserts: (1) the main insert that is comprised of a functional *cp4 epsps* cassette, and (2) an additional insert that is comprised of a 72 bp segment of the *cp4 epsps* coding sequence.

The GMO Panel has previously assessed soybean event 40-3-2 as a single event and as part of a two-event soybean stack submitted by Pioneer Hi-Bred International Inc (see Table 1).

Table 1: EFSA GMO Panel scientific opinions on soybean event 40-3-2

Event	Application	EFSA Scientific Opinions
40-3-2	EFSA-GMO-RX-40-3-2 _[8-1a/20-1a] ;[8-1b/20-1b] EFSA-GMO-NL-2005-24	EFSA GMO Panel (2010a) EFSA GMO Panel (2012)
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 22 September 2016, Monsanto Company sent to the European Commission new sequencing information relating to soybean event 40-3-2, on the basis of Articles 9 and 21 of Regulation (EC) 1829/2003. On 4 April 2017, the European Commission requested the European Food Safety Authority (EFSA) to evaluate the data and analyses provided by Monsanto and indicate whether, on the basis of these elements, the conclusions of adopted opinions for soybean 40-3-2 as a single event or as part of the stacked event have to be adapted. Subsequently, the EFSA has evaluated the data and methodology provided for soybean event 40-3-2 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 event 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.

2.2.1. Sequence information previously submitted to EFSA for soybean 40-3-2

The applicant previously submitted information on the sequence of soybean event 40-3-2, as part of applications EFSA-GMO-RX-40-3-2_[8-1a/20-1a], EFSA-GMO-RX-40-3-2_[8-1b/20-1b] and EFSA-GMO-NL-2005-24 (EFSA GMO Panel, 2010a, 2012). Soybean event 40-3-2 contains an insert, consisting of the *cp4 epsps* expression cassette and an additional insert consisting only of 72 bp of the *cp4 epsps* coding sequence.

2.2.2. New information for soybean event 40-3-2 submitted as part of the current mandate

The applicant recently re-sequenced the soybean event 40-3-2 using the 305423 × 40-3-2 soybean stack¹ material and compared this sequence with the soybean event sequence originally reported in the context of the single soybean event 40-3-2 (EFSA GMO Panel, 2010a, 2012) and the stacked soybean 305423 × 40-3-2 (EFSA GMO Panel, 2016). This revealed an additional adenosine (A) at position 332 [ACACG] in the 5' flanking region of the 72 bp additional insert of *cp4 epsps* coding sequence, relative to the originally reported sequence in the context of the single soybean event

¹ Additional information: Study # REG-2016-0257.

40-3-2 and the stacked soybean 305423 × 40-3-2. The applicant re-examined the raw sequencing data for soybean event 40-3-2,² and reported that this additional nucleotide was already present in the original plant material used for the risk assessment of soybean event 40-3-2, but was unintentionally omitted in the reports provided to EFSA.

For the reported difference, the applicant evaluated the impact on the original bioinformatics analyses.³ The addition of an 'A' in the updated sequence was found to impact the outcome of the analysis of the open reading frames (ORFs) spanning the junctions between the additional insert region and soybean genomic DNA.⁴ Therefore, EFSA requested the applicant to perform new bioinformatics for any effected ORFs for similarity to known allergens or toxins using the updated sequence.

The applicant performed a new analysis of the junction ORFs and documented that only one putative ORF (72_5b) at the 5' junction of the 72 bp additional insert was affected.⁴ The results of the updated bioinformatics for this ORF were processed and a risk assessment was provided.

3. Assessment

The provided data showed a sequence difference in event 40-3-2 in the stacked soybean 305423 × 40-3-2, compared to what was previously reported to EFSA in the context of the single soybean event 40-3-2 and the stacked soybean 305423 × 40-3-2 (EFSA GMO Panel, 2010a, 2012, 2016).

Bioinformatic analyses performed with the updated sequence of the affected ORF (72_5b) at the 5' junction of the 72 bp additional CP4 EPSPS insert with regard to potential similarity with allergens or toxins, were considered relevant for the current assessment. The bioinformatics searches for similarity to allergens were performed according to EFSA guidelines (EFSA GMO Panel, 2010b, 2011). Results indicate that the ORF containing the reported difference does not show similarity with known allergens or toxins.

The other studies performed for the risk assessment of soybean event 40-3-2 in the context of the single soybean event 40-3-2 and the stacked soybean 305423 × 40-3-2 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 40-3-2 as present in the stacked soybean 305423 × 40-3-2 contains an additional nucleotide. Re-examination of the original sequencing data of the single soybean event 40-3-2 by the applicant, indicated that this additional nucleotide was already present in the original plant material used for the risk assessment of soybean event 40-3-2. The bioinformatic analyses 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, based on the information provided, that the original risk assessment of soybean event 40-3-2 as a single and as a part of the stacked soybean 305423 × 40-3-2 remains valid.

Documentation provided to EFSA

- 1) Letter from the European Commission, received on 4 April 2017, concerning a request to analyse new sequencing information for soybean event 40-3-2.
- 2) Acknowledgement letter dated 5 May 2017 from EFSA to the European Commission.
- 3) Letter from EFSA to the applicant dated 11 May 2017 requesting additional information.
- 4) Letter from the applicant to EFSA dated 31 May 2017 submitting additional information.
- 5) Letter from EFSA to the European Commission dated 26 June 2017 for status update.

² Additional information: 31 May 2017; Report MSL0028011.

³ Scientific information: Report RAR-2016-0416.

⁴ Additional information: 31 May 2017.

References

- EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010a. Scientific Opinion on applications (EFSA-GMO-RX-40-3-2[8-1a/20-1a], EFSA-GMO-RX-40-3-2[8-1b/20-1b]) for renewal of authorisation for the continued marketing of (1) food containing, consisting of, or produced from genetically modified soybean 40-3-2; (2) feed containing, consisting of, or produced from soybean 40-3-2; (3) other products containing or consisting of soybean 40-3-2 with the exception of cultivation, all under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 2010;8(12):1908, 38 pp. <https://doi.org/10.2903/j.efsa.2010.1908>
- EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010b. Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal 2010;8(7):1700, 168 pp. <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>
- EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2012. Scientific Opinion on an application (EFSA-GMO-NL-2005-24) for the placing on the market of the herbicide tolerant genetically modified soybean 40-3-2 for cultivation under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 2012;10(6):2753, 110 pp. <https://doi.org/10.2903/j.efsa.2012.2753>
- 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

A	adenosine
bp	base pair
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
GM	genetically modified
GMO	genetically modified organisms
ORF	open reading frame