

TECHNICAL REPORT

Report on the safety analysis of oilseed rape Ms8 x Rf3 pollen in food or as food¹

European Food Safety Authority^{2, 3}

European Food Safety Authority (EFSA), Parma, Italy

SUMMARY

Following a request from the European Commission, EFSA was asked to assess the safety of oilseed rape Ms8 x Rf3 pollen as food or in food. The EFSA scientific analysis was performed taking into consideration the following information: i) the EFSA GMO Panel scientific opinion on oilseed rape Ms8 x Rf3 published in 2005; ii) the EFSA GMO Panel scientific opinion for renewal of oilseed rape Ms8 x Rf3 published in 2009; and iii) a scientific literature search.

Oilseed rape Ms8 x Rf3 expresses the Barnase, Barstar and PAT proteins. The Barnase and Barstar proteins are both under the control of tapetum-specific promoter and their expression is highly restricted spatially and temporally. The *bar* coding sequence, coding for the PAT protein, is under a promoter which is active in all green tissues and in pollen. The stability of the transgene inserts in Ms8 and Rf3 has been demonstrated over multiple generations, implying that the integrity of the inserts was maintained throughout microsporogenesis and pollen production. Bioinformatic analyses of the putative translation products of open reading frames spanning the 5' and 3' junction regions of the inserts did not reveal significant similarity to known allergens or toxins. In laboratory experiments, the Barnase and Barstar proteins could not be detected in the pollen. The PAT protein expression was detected in pollen, but the level was not quantified. A search in the open scientific literature did not yield any data on the level of PAT in oilseed rape Ms8xRf3 pollen.

For the food and feed safety assessment, the EFSA GMO Panel previously assessed the safety of the Barnase, Barstar and PAT proteins in oilseed rape Ms8 x Rf3. Updated bioinformatics studies indicated that no similarities exist between the newly expressed proteins Barnase, Barstar and PAT and known toxic proteins or allergens. The EFSA GMO Panel assessed the safety of the PAT protein not only on oilseed rape, but also in other crops and did not identify any issue regarding its potential toxicity and allergenicity.

Additionally, EFSA considered the possibility that unintended effects of the genetic modification might have occurred in oilseed rape Ms8 x Rf3 pollen. Based on the information analysed in this report, there is no indication for unintended effects due to the genetic modification. The EFSA GMO Panel previously concluded that oilseed rape Ms8 x Rf3 is as safe and as nutritious as the non-GM counterpart, in the context of its intended uses.

¹ On request from European Commission, Question No EFSA-Q-2012-00110, issued on 8 February 2012.

² Correspondence: gmo@efsa.europa.eu

³ Acknowledgement: EFSA wishes to thank EFSA staff: Antonio Fernández Dumont and Zoltán Divéki for the support provided to this output.

Suggested citation: European Food Safety Authority; Report on the safety analysis of oilseed rape Ms8 x Rf3 pollen in food or as food. Supporting Publications 2012:EN-228. [6 pp.]. Available online: <http://www.efsa.europa.eu/en/publications/supporting.htm>

Considering the available data described in this report, no indication of potential concerns over the safety of the newly expressed Barnase, Barstar and PAT proteins, nor the occurrence of unintended effects in oilseed rape Ms8 x Rf3 pollen that could raise safety concerns, have been identified.

KEY WORDS

GMO, oilseed rape, Ms8 x Rf3, herbicide tolerant, food and feed safety, risk assessment, pollen

© European Food Safety Authority, 2012

TABLE OF CONTENTS

Summary 1
Table of contents 3
Background 4
Terms of reference 4
Assessment 5
1. Introduction 5
2. Molecular Characterisation..... 5
3. Food and feed safety assessment 5
Documentation provided to EFSA 6
References 6

BACKGROUND

On 24 November 2011, EFSA received a request from the European Commission for scientific assistance on the evaluation of the safety of pollen produced by oilseed rapes GT73 and Ms8 x Rf3. More specifically, EFSA was requested to “*assess, pursuant to Article 31 of Regulation (EC) No 178/2002, the safety of GT73 and Ms8xRf3 pollen as food or in food*” (SANCO/E1/SP/mb Ares (2011) 1361619).

EFSA addressed this request by providing two separate technical reports, focusing on oilseed rape Ms8 x Rf3 pollen (this report) and on oilseed rape GT73 pollen (EFSA, 2012).

For oilseed rape Ms8 x Rf3, EFSA analysed data sets available in the context of two previously finalised opinions of the EFSA GMO Panel on oilseed rape Ms8xRf3 (EFSA 2005, 2009). Additionally, EFSA performed a scientific literature search on oilseed rape Ms8 x Rf3 and derived food and feed relevant for the safety assessment.

EFSA is currently in the process of assessing an application submitted under Regulation (EC) No 1829/2003 covering the use of oilseed rape Ms8 x Rf3 as food or in food (EFSA-GMO-BE-2010-81). The scope of this application also includes the use of pollen. The data sets in this dossier cannot yet be considered complete, since the working groups of the EFSA GMO Panel are currently reviewing the application and have identified questions to the applicant. Consequently, the data sets in the dossier EFSA-GMO-BE-2010-81 are not considered in this report.

TERMS OF REFERENCE

EFSA was requested by European Commission to “*assess, pursuant to Article 31 of Regulation (EC) No 178/2002, the safety of GT73 and Ms8xRf3 pollen as food or in food*”.

ASSESSMENT

1. Introduction

In this technical report, the EFSA scientific analysis was performed taking into consideration the following information: i) the initial EFSA GMO Panel scientific opinion on oilseed rape Ms8 x Rf3 (EFSA, 2005); ii) the EFSA GMO Panel scientific opinion for renewal of oilseed rape Ms8 x Rf3 (EFSA, 2009); and iii) a scientific literature search on oilseed rape Ms8 x Rf3 and derived food and feed relevant for the safety assessment.

2. Molecular Characterisation

Oilseed rape Ms8 x Rf3 was developed by conventional breeding techniques from Ms8 (male sterile) and Rf3 (restorer of fertility) oilseed rape lines. While Ms8 expresses the Barnase and PAT proteins, Rf3 expresses the Barstar and the PAT proteins. The Barnase and Barstar proteins are both under the control of tapetum-specific promoter and their expression is highly restricted spatially and temporally. The *bar* coding sequence, coding for the PAT protein, is under a promoter which is active in all green tissues and in pollen. The stability of the transgene inserts in Ms8 and Rf3 has been demonstrated over multiple generations, implying that the integrity of the inserts was maintained throughout microsporogenesis and pollen production (EFSA, 2005). Bioinformatic analyses of the putative translation products of open reading frames spanning the 5' and 3' junction regions of the inserts did not reveal significant similarity to known allergens or toxins (EFSA, 2009).

In laboratory experiments, the Barnase and Barstar proteins could not be detected in the pollen (EFSA-GMO-RX-Ms8-Rf3, Technical dossier, p. 59). The PAT protein expression was detected in pollen, but the level was not quantified. However, since the PAT mRNA was not detected in pollen by northern analysis, it could be inferred that the level of the PAT protein in pollen is likely to be very low. In oilseed rape Ms8 x Rf3 seed, the PAT level ranged between 0.11 – 0.22 µg/g fresh weight (EFSA-GMO-RX-Ms8-Rf3, Technical dossier, p. 60). A search in the open scientific literature did not yield any data on the level of PAT in oilseed rape Ms8xRf3 pollen.

3. Food and feed safety assessment

For the preparation of this section on the food/feed safety assessment of oilseed rape Ms8 x Rf3 pollen as food or in food, EFSA considered both the safety assessment of the newly expressed proteins as well as the possibility of unintended effects due to the genetic modification.

In relation to the newly expressed proteins, Barnase and Barstar are only expressed in the tapetum cells of the flower buds. In line with this, the results of western blot analyses indicate that Barnase and Barstar are not detected in pollen (EFSA-GMO-RX-Ms8-Rf3). On the other hand, PAT protein expression was detected in pollen, but the level was not quantified. Updated bioinformatics studies indicated that no similarities exist between the newly expressed proteins Barnase, Barstar and PAT and known toxic proteins or allergens (EFSA, 2009). Furthermore, the EFSA GMO Panel has assessed the safety of the PAT protein not only on oilseed rape (EFSA, 2005; 2009) but also in other crops and did not identify any issue regarding potential toxicity and allergenicity of the protein (e.g. EFSA-GMO-NL-2004-02, EFSA-GMO-UK-2004-04, EFSA-GMO-NL-2005-12, EFSA-GMO-NL-2005-13, EFSA-GMO-NL-2005-18, EFSA-GMO-UK-2005-25, etc). Based on the molecular characterisation analysis, there is no reason to expect that the newly expressed protein expressed in oilseed rape pollen would be different than in other parts of the plant. Therefore, these toxicity and allergenicity data sets are also applicable to the newly expressed protein in pollen.

Additionally, EFSA considered the possibility that unintended effects of the genetic modification might have occurred in oilseed rape Ms8 x Rf3 pollen. Possible unintended effects are identified by the molecular characterisation and the comparative analysis of compositional, agronomic and phenotypic characteristics of the GM crop versus its non-GM comparator.

For oilseed rape Ms8 x Rf3 grain, the EFSA GMO Panel previously concluded that there is no indication for unintended effects due to the genetic modification (EFSA, 2005). No information is available on the composition of oilseed rape Ms8 x Rf3 pollen or its conventional counterpart in the dossiers submitted to EFSA. In relation to the agronomic and phenotypic analysis, the EFSA GMO Panel concluded that agronomic performance was not affected except for higher yield due to hybrid vigour (EFSA, 2005).

Unintended effects of the whole crop were also addressed in the frame of allergenicity assessment. The EFSA GMO Panel concluded that there is no evidence that the overall allergenicity of the GM crop has changed due to any unintended effect (EFSA, 2005). This conclusion is also supported by an *in silico* analysis on epitope homology, which indicates that the putative translation products of the open reading frames spanning the 5' and 3' junction regions of the insert did not reveal significant similarity to known allergens (EFSA, 2009).

A broiler feeding study confirmed that oilseed rape Ms8 x Rf3 is nutritionally equivalent to its non-GM counterpart (EFSA, 2009).

A scientific literature search of peer-reviewed scientific data on oilseed rape Ms8 x Rf3 and derived food and feed was performed. Detailed analysis showed that either they had already been taken into consideration by the GMO Panel in its previous opinions, or contained no information relevant for the safety assessment.

CURRENT CONCLUSIONS

At present and considering the available data described above, no indication of potential concerns over the safety of the newly expressed Barnase, Barstar and PAT proteins, nor the occurrence of unintended effects in oilseed rape Ms8 x Rf3 pollen have been identified that could raise safety concerns.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the European Commission, received on 24 November 2011, concerning a request for a scientific assistance on the safety of pollen produced by GT73 and Ms8xRf3 oilseed rapes.

REFERENCES

- EFSA (European Food Safety Authority), 2005. Opinion of the Scientific Panel on genetically modified organisms [GMO] related to the application (Reference C/BE/96/01) for the placing on the market of glufosinate-tolerant hybrid oilseed rape Ms8 x Rf3, derived from genetically modified parental lines (Ms8, Rf3), for import and processing for feed and industrial uses, under Part C of Directive 2001/18/EC from Bayer CropScience. The EFSA Journal 281, 1-23.
- EFSA (European Food Safety Authority), 2009. Scientific Opinion on an application (EFSA-GMO-RX-MS8-RF3) for renewal of the authorisation for continued marketing of existing (1) food and food ingredients produced from genetically modified glufosinate-tolerant oilseed rape Ms8, Rf3 and Ms8 x Rf3, and (2) feed materials produced from genetically modified glufosinate-tolerant oilseed rape Ms8, Rf3 and Ms8 x Rf3, under Regulation (EC) No 1829/2003 from Bayer CropScience. The EFSA Journal 2009; 7(9):1318.
- EFSA (European Food Safety Authority), 2012. Report on the safety analysis of oilseed rape GT73 pollen in food or as food. Supporting publications 2012:EN-227.