SCIENTIFIC OPINION

Scientific Opinion on the revised molecular characterisation for Rf3 oilseed rape received from the Competent Authority of Belgium under Article 20(3) of Directive 2001/18/EC[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The DG Environment of the European Commission has requested the EFSA Panel on Genetically Modified Organisms to deliver a scientific opinion on the revised molecular characterisation for Rf3 oilseed rape received from the Competent Authority of Belgium under Article 20(3) of Directive 2001/18/EC. The GMO Panel was asked to consider whether the new information received in respect of oilseed rape Rf3 could have consequences for the risks of this GMO to human health or the environment within the scope of Directive 2001/18/EC. In particular, EFSA was requested to take account of the objections raised by the Competent Authority of Austria in this context. Based on the revised molecular characterisation and on the additional information submitted in the frame of assessment of application EFSA-GMO-RX-Ms8-Rf3, the GMO Panel concludes that (1) the new information received on oilseed rape Rf3 does not have consequences for the risks of this GMO to human health or the environment within the scope of Directive 2001/18/EC and (2) the points raised by the Competent Authority of Austria are addressed in the opinion of the EFSA GMO Panel on application EFSA-GMO-RX-Ms8-Rf3.

KEY WORDS

Rf3, Ms8 x Rf3, oilseed rape, updated molecular characterisation, safety, risk assessment, 2001/18/EC

1 On request from the European Commission, Question No EFSA-Q-2009-00956, adopted on 13 April 2010.

2 Panel members: Hans Christer Andersson, Salvatore Arpaia, Detlef Bartsch, Josep Casacuberta, Howard Davies, Patrick du Jardin, Gerhard Flachowsky, Lieve Herman, Huw Jones, Sirpa Kärenlampi, Jozsef Kiss, Gijs Kleter, Harry Kuiper, Antoine Messéan, Kaare Magne Nielsen, Joe Perry, Annette Pöting, Jeremy Sweet, Christoph Tebbe, Atte Johannes von Wright, and Jean-Michel Wal. Opinion is shared by all members of the Panel. 0 Panel member(s) with minority opinion. 0 members of the Panel did not participate in [part of] the discussion on the subject referred to above because of potential conflicts of interest identified in accordance with the EFSA policy on declarations of interests. Correspondence: gmo@efsa.europa.eu

3 Acknowledgement: The Panel wishes to thank the members of the Molecular Characterisation Working Group for the preparation of this opinion and EFSA's staff member Zoltán Divéki for the support provided to this EFSA scientific output.

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SUMMARY

Following the request from the DG Environment of the European Commission on 4 December 2009, the EFSA Panel on Genetically Modified Organisms was asked to deliver a scientific opinion on the revised molecular characterisation for Rf3 oilseed rape received from the Competent Authority of Belgium under Article 20(3) of Directive 2001/18/EC. In particular, the GMO Panel was asked to consider "Whether the new information received in respect of oilseed rape Rf3, Ms8 x Rf3 could have consequences for the risks of the GMO to human health or the environment within the scope of Directive 2001/18/EC. In particular, EFSA is requested to take account of the objections raised by the Competent Authority of Austria in this context."

The GMO Panel had already considered the revised molecular characterisation of event Rf3 as well as additional information provided in response to a request from the GMO Panel in delivering its scientific opinion on application EFSA-GMO-RX-Ms8-Rf3. The GMO Panel concluded on 9 September 2009 that "oilseed rape Ms8, Rf3 and Ms8 x Rf3 is unlikely to have an adverse effect on human and animal health or, in the context of its proposed uses, on the environment".

Upon receipt of the current mandate, the GMO Panel has scrutinised the objections raised by the Competent Authority of Austria as well as the additional information provided by Bayer Crop Science in response to a request from the Competent Authority of Belgium.

The GMO Panel is of the opinion that (1) the new information received on oilseed rape Rf3 does not have consequences for the risks of this GMO to human health or the environment within the scope of Directive 2001/18/EC and (2) the points raised by the Competent Authority of Austria are addressed in the recent opinion of the EFSA GMO Panel on application EFSA-GMO-RX-Ms8-Rf3 for renewal of the authorisation for continued marketing of existing food, food ingredients and feed materials produced from oilseed rape Ms8, Rf3 and Ms8 x Rf3 under Regulation 1829/2003.

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

In May 2009, the Commission received new information in respect of oilseed rape Rf3 from the Competent Authority (CA) of Belgium (BE), which is the lead Member State for this application (C/BE/96/01) under Directive 2001/18/EC.

In summary, the notifier, Bayer Crop Science discovered that the previous molecular characterisation of the Rf3 event was not correct and provided a revised molecular characterisation. This information was considered new information made available after the consent, which could have consequences for the *"risks of the GMO to human health or the environment"*. It was therefore examined under the procedure of Article 20(3) of Directive 2001/18/EC. The provisions of this article were thereby applied and the BE CA provided an assessment report and further additional information originally provided by the notifier. In accordance with the requirements of Directive 2001/18/EC, the notification was then transmitted to the competent authorities of other Member States and the Austrian CA responded by raising a reasoned objection during the statutory 60-day period.

The objection raised by Austria was forwarded to Bayer Crop Science on 14 October 2009 with a request to provide the Commission with a response in accordance with Article 18(1) of Directive 2001/18/EC.

Bayer Crop Science duly responded on 13 November 2009 and indicated that the EFSA GMO Panel considered the information on the revised molecular characterisation of Rf3 oilseed rape in its opinion on EFSA-GMO-RX-Ms8-Rf3 published in September 2009. In accordance with the procedure set out under Article 18(1) of Directive 2001/18/EC and notwithstanding the information provided by Bayer Crop Science, the Commission was required to consult EFSA.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA is requested under Article 18 of Directive 2001/18/EC and in conjunction with Articles 29(1) and 22(5)(c) of Regulation (EC) No 178/2002, to provide a scientific opinion within 90 days from the reception of this mandate as to whether:

- the new information received in respect of oilseed rape Rf3, Ms8 x Rf3 could have consequences for the risks of the GMO to human health or the environment within the scope of Directive 2001/18/EC;

- in particular, EFSA is requested to take account of the scientific objections raised by the Competent Authority of Austria in this context, to highlight diverging views, if any, and how these are resolved in the opinion;

- where the aforementioned aspects have been considered in EFSA's recent opinion on application EFSA-GMO-RX-Ms8-Rf3 for renewal of the authorisation for continued marketing of existing (l) food and food ingredients produced from GM glufosinate-tolerant oilseed rape Ms8, Rf3 and Ms8 x Rf3 and (2) feed materials produced from GM glufosinate-tolerant oilseed rape Ms8, Rf3 and Ms8 x Rf3 under Regulation 1829/2003 from Bayer Crop Science, it will suffice to cross-reference that opinion.

ASSESSMENT

1. Question from DG Environment on whether the new information received in respect of oilseed rape Rf3 could have consequences for the risks of this GMO to human health or the environment within the scope of Directive 2001/18/EC

In its opinion on the application EFSA-GMO-RX-Ms8-Rf3 (EFSA, 2009), the EFSA GMO Panel has taken into consideration the updated molecular characterisation of event Rf3 as well as additional information submitted following a request from the GMO Panel. The GMO Panel has concluded that "These updated molecular and bioinformatic analyses provided for oilseed rape Ms8 and Rf3 as well as additional data, provided upon request of the EFSA GMO Panel, do not indicate any concerns.", and that therefore "oilseed rape Ms8, Rf3 and Ms8 x Rf3 are unlikely to have any adverse effect on human and animal health or, in the context of their proposed uses, on the environment."

2. Objections by the Competent Authority of Austria and cross-references to the EFSA opinion on application EFSA-GMO-RX-Ms8-Rf3

2.1. General remarks

The revised information on the molecular characterisation of Rf3 oilseed rape submitted to the EFSA GMO Panel for the application EFSA-GMO-RX-Ms8-Rf3 on 3 February 2009 included updated bioinformatic analyses, additional Southern and PCR analyses and an updated analysis of the complete DNA sequence of the Rf3 insert. The updated bioinformatic analyses included in the revised molecular characterisation of Rf3 were based on the updated insert structure and did not indicate any safety concerns. Following an additional request from the GMO Panel (12 March 2009, Ref. PB/SM/md (2009) 3777062), the applicant:

- clarified that sequencing of the pTHW107 and pTHW118 transformation vectors revealed some differences compared to the formerly deduced putative sequences, but that none of the differences between the deduced sequence and the determined sequence were located in the functional regions subsequently transferred to the recipient oilseed rape;
- provided evidence that no DNA rearrangements have occurred in the Rf3 insertion site compared to the original submission.

The GMO Panel has concluded that the differences between the original and the new molecular characterisations of event Rf3 are not due to different molecular structures but to an alternative interpretation of the originally available data. The new molecular characterisation combined with the additional data requested by the GMO Panel allowed for the unambiguous interpretation of the molecular structures.

2.2. Specific remarks

Comments regarding the original molecular characterisation of event Rf3 (Comments 8, 9a and 10c of the Competent Authority of Austria)

This issue is addressed in Chapter 3.1, paragraph 2 of EFSA's opinion on application EFSA-GMO-RX-Ms8-Rf3: "The EFSA GMO Panel received an updated description of the insert structure in the Rf3 line. As the original analyses could lead to two different interpretations of the structure of the insert, the applicant performed additional Southern and PCR analyses to distinguish between the two models." The revised structure of event Rf3 is supported by the nucleotide sequence analysis of the complete Rf3 insert.

Comments regarding the genetic stability of event Rf3 (Comments 9b and 11 of the Competent Authority of Austria)

This issue is addressed in Chapter 3.1, paragraphs 1 and 2 of EFSA's opinion on application EFSA-GMO-RX-Ms8-Rf3:

"Updated annotation of the plasmid vectors pTHW107 and pTHW118 based on the determined nucleotide sequence was provided in the renewal application. On further request, the applicant clarified that sequencing of the plasmids revealed some differences compared to the formerly deduced putative sequences. As none of the differences between the deduced sequence and the determined sequence is located in the functional regions subsequently transferred to the recipient oilseed rape, they are not considered relevant to the current as well as previous risk assessments."

"Compared to the originally reported insert structure the region spanning the complete and the truncated Pta29 promoter elements is inverted. The data were supported by the analysis of the complete DNA sequence of the Rf3 insert. Southern analysis with identical restriction enzyme and probe combination indicated that the structure of the insert in the plant was not changed since the original notification (reference C/BE/96/01). The EFSA GMO Panel accepts the revised interpretation of the insert structure and considers that it does not indicate instability of the insert in the plant."

Comments regarding the analysis of cryptic ORFs (Comments 10a, 10b, 13 and 14 of the Competent Authority of Austria) and the analysis of the genomic sequences flanking the Rf3 insert (Comment 12 of the Competent Authority of Austria)

These issues are addressed in Chapter 3.1, paragraph 3 of EFSA's opinion on application EFSA-GMO-RX-Ms8-Rf3: "At the request of the EFSA GMO Panel, the applicant performed new bioinformatic analyses. These cover all junctions created by the transformation process, including those flanking the genomic sequences as well as the internal junctions in the Rf3 event (revised insert structure). Possible interruptions of endogenous genes were analyzed. Putative new open reading frames (ORFs; from stop to stop codon) were compared to known toxins and allergens. The analyses did not reveal any newly created ORFs with significant identity to known toxins or allergens. The data provided do not indicate any safety concerns with regard to the interruption of known genes or regulatory elements or from the potential production of new toxins or allergens." Furthermore, the nucleotide sequence of the pre-insertion locus has been determined in wild-type *Brassica napus* (De Beuckeleer et al., 1995, page 48), which provides unequivocal evidence that the transgenic event is inserted into a *Brassica napus* genome.

The GMO Panel follows a weight of evidence approach in risk assessment and the molecular characterisation is supported by data from other analyses including agronomic performance, compositional analysis and nutritional properties (EFSA, 2005). None of these raised any safety concern.

CONCLUSIONS

The EFSA GMO Panel concludes that (1) the new information received on oilseed rape Rf3 does not have consequences for the risks of this GMO to human health or the environment within the scope of Directive 2001/18/EC and (2) the points raised by the Competent Authority of Austria are addressed in the recent opinion of the EFSA GMO Panel on application EFSA-GMO-RX-Ms8-Rf3 for renewal of the authorisation for continued marketing of existing food, food ingredients and feed materials produced from oilseed rape Ms8, Rf3 and Ms8 x Rf3 under Regulation 1829/2003.

DOCUMENTATION PROVIDED TO EFSA

1. Letter and CD-ROM from the European Commission*, dated 4 December 2009, concerning a request to consider "Whether the new information received in respect of oilseed rape Rf3, Ms8 x Rf3 could have consequences for the risks of the GMO to human health or the environment within

the scope of Directive 2001/18/EC. In particular, EFSA is requested to take account of the objections raised by the Competent Authority of Austria in this context." The letter included the following attachments:

a) A letter from Bayer Crop Science to the Competent Authority of Belgium providing them with revised information on the molecular characterisation for the Rf3 insert.*

b) Advice of the Belgian Biosafety Advisory Council on the revised molecular characterisation of Rf3 oilseed rape submitted by Bayer Crop Science.*

c) *Brassica napus*, transformation event Rf3, *in silico* analysis of putative ORF sequences for identifying potential homologies to known toxins and allergens. Capt, 2008a.

d) *Brassica napus*, transformation event Rf3, *in silico* analysis of putative ORF sequences for identifying potential homologies to known toxins and allergens. Capt, 2008b.

e) Update of the bioinformatics analysis of the integration sequences from oilseed rape transformation event Rf3, De Pestel, 2008.

f) Bioinformatics analysis of newly created ORFs coding for minimum 3 amino acids from oilseed rape transformation event Rf3, Debaveye 2008.

g) Detailed insert characterization of *Brassica napus* transformation event Rf3, Moens and Criel, 2008.

h) Bayer Crop Science response (November 2009).*

i) EFSA opinion 2009 on renewal application EFSA-GMO-RX-Ms8-Rf3.

j) Update of the description of the transgenic locus of *Brassica napus* transformation event Rf3, Moens, 2008 (confidential).

k) Rf3 breeding diagram (confidential).

l) Reasoned objection received from the Competent Authority of Austria.*

2. Acknowledgement of receipt of a letter, dated 21 December 2009, from EFSA to the European Commission. The letter also included a request to provide EFSA with the files missing from the datapackage.

3. Letter and CD-ROM from the European Commission, dated 21 January 2010, including the complete datapackage.

4. Letter from the EFSA, dated 23 February 2010, acknowledging the reception of the complete datapackage and asking for the confirmation of the proposed deadline to produce a scientific opinion on this matter.

* These documents can be retrieved from the EFSA register of questions.

REFERENCES

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allergens. Report ID: M-326808-01-1. Additional information to the technical dossier of application EFSA-GMO-RX-Ms8-Rf3.

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Debaveye 2008. Bioinformatics analysis of newly created ORFs coding for minimum 3 amino acids from oilseed rape transformation event Rf3. Report ID: BIO3-004_Bioinfo_189. Additional information to the technical dossier of application EFSA-GMO-RX-Ms8-Rf3.

EFSA, 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. Available online: www.efsa.europa.eu

EFSA, 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. EFSA Journal 2009; 7(9):1318 [12 pp.]. doi:10.2903/j.efsa.2009.1318. Available online: www.efsa.europa.eu

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Moens and Criel, 2008. Detailed insert characterization of *Brassica napus* transformation event Rf3. Report ID: BBS07-001. Additional information to the technical dossier of application EFSA-GMO-RX-Ms8-Rf3.