**SCIENTIFIC OPINION**

# Scientific Opinion on application (EFSA-GMO-BE-2010-81) for the placing on the market of genetically modified herbicide-tolerant oilseed rape Ms8, Rf3 and Ms8 × Rf3 for food containing or consisting of, and food produced from or containing ingredients produced from, oilseed rape Ms8, Rf3 and Ms8 × Rf3 (with the exception of processed oil) under Regulation (EC) No 1829/2003 from Bayer[1]

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

European Food Safety Authority (EFSA), Parma, Italy

## ABSTRACT

This scientific opinion is a risk assessment for the placing on the market of the genetically modified (GM) herbicide-tolerant oilseed rape (OSR) Ms8, Rf3 and Ms8×Rf3 for food containing or consisting of, and food produced from or containing ingredients produced from, these GM plants. OSR Ms8 (male sterile) and Rf3 (fertility restorer) are the parents of OSR Ms8×Rf3, which is fertile, contains the *bar*, *barstar* and *barnase* genes, and is tolerant to glufosinate-ammonium-containing herbicides. Integrity of the inserts present in the single events was demonstrated in the stack. Molecular characterisation did not reveal any safety issues. No biologically relevant differences were identified in the composition or agronomic and phenotypic characteristics of OSR Ms8×Rf3, as compared with its non-GM comparator, except for the newly expressed proteins. The safety assessment identified no concerns regarding the potential toxicity and allergenicity of OSR Ms8, Rf3 and Ms8×Rf3. A broiler study confirmed that OSR Ms8×Rf3 is as nutritious as its non-GM comparator. There are no indications of an increased likelihood of establishment and spread of feral OSR Ms8, Rf3 and Ms8×Rf3 plants, or of hybridising wild relatives, unless exposed to glufosinate-ammonium-containing herbicides. Considering the intended uses, potential interactions of feral OSR Ms8, Rf3 and Ms8×Rf3 plants with the biotic and abiotic environment are not considered an issue. Environmental risks associated with a possible horizontal transfer into bacteria have not been identified. The monitoring plan and reporting intervals are in line with the intended uses. In conclusion, the EFSA GMO Panel considers that the information available for OSR Ms8, Rf3 and Ms8×Rf3 addresses the scientific comments raised by Member States and that OSR Ms8, Rf3 and Ms8×Rf3 are unlikely to have an adverse effect on human and animal health or on the environment, in the context of their intended uses.



[1] On request from the Competent Authority of Belgium for an application (EFSA-GMO-BE-2010-81) submitted by Bayer, Question No EFSA-Q-2010-00947, adopted on 6 September 2012.

[2] Panel members: Salvatore Arpaia, Andrew Nicholas Edmund Birch, Andrew Chesson, Patrick du Jardin, Achim Gathmann, Jürgen Gropp, Lieve Herman, Hilde-Gunn Hoen-Sorteberg, Huw Jones, Jozsef Kiss, Gijs Kleter, Pagona Lagiou, Martinus Lovik, Antoine Messéan, Hanspeter Naegeli, Kaare Magne Nielsen, Jaroslava Ovesna, Joe Perry, Nils Rostoks, Christoph Tebbe. Correspondence: gmo@efsa.europa.eu

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**KEY WORDS**

GMO, oilseed rape, Ms8, Rf3, Ms8 × Rf3, herbicide tolerant, human and animal health, food, Regulation (EC) No 1829/2003

## SUMMARY

Following the submission of an application (EFSA-GMO-BE-2010-81) under Regulation (EC) No 1829/2003[4] from Bayer, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) was asked to deliver a scientific opinion on the safety of genetically modified (GM) herbicide-tolerant oilseed rape Ms8 (Unique Identifier ACS-BNØØ5-8), Rf3 (Unique Identifier ACS-BNØØ3-6) and Ms8 × Rf3 (Unique Identifier ACS-BNØØ5-8 × ACS-BNØØ3-6) for food containing or consisting of, and food produced from or containing ingredients produced from, oilseed rape Ms8, Rf3 and Ms8 × Rf3 (with the exception of processed oil).

In delivering its scientific opinion, the EFSA GMO Panel considered the application EFSA-GMO-BE-2010-81, additional information supplied by the applicant, scientific comments submitted by the Member States, and relevant scientific publications. Furthermore, relevant information from previous applications for the placing on the European Union market of oilseed rape Ms8, Rf3 and Ms8 × Rf3 was taken into account. The scope of application EFSA-GMO-BE-2010-81 is for food containing or consisting of, and food produced from or containing ingredients produced from, oilseed rape Ms8, Rf3 and Ms8 × Rf3 (with the exception of processed oil) within the EU, as for any non-GM oilseed rape, but excludes cultivation in the EU. The EFSA GMO Panel evaluated oilseed rape Ms8, Rf3 and Ms8 × Rf3 with reference to the intended uses and appropriate principles described in its guidance documents for the risk assessment of GM plants and derived food and feed (EFSA, 2006a), for the risk assessment of GM plants containing stacked transformation events (EFSA, 2007a) and on the post-market environmental monitoring (PMEM) of GM plants (EFSA, 2006b, 2011a). The scientific risk assessment included molecular characterisation of the inserted DNA and expression of the corresponding proteins. An assessment of the comparative analysis of composition and phenotypic and agronomic characteristics was undertaken, and the safety of the new proteins and the whole food was evaluated with respect to potential toxicity, allergenicity and nutritional wholesomeness. An assessment of the environmental impacts and PMEM plan were undertaken.

Oilseed rape Ms8, Rf3 and Ms8 × Rf3 have been the subject of earlier risk assessments by the EFSA GMO Panel with the scope covering: (i) import and processing, and feed and industrial uses in 2005; and (ii) the renewal of the authorisation for continued marketing of existing food and food ingredients produced from oilseed rape Ms8, Rf3 and Ms8 × Rf3, and feed materials produced from oilseed rape Ms8, Rf3 and Ms8 × Rf3 in 2009. The EFSA GMO Panel concluded that oilseed rape Ms8, Rf3 and Ms8 × Rf3 are unlikely to have an adverse effect on human and animal health or, in the context of its proposed uses, on the environment. In addition, EFSA recently published a technical report on a safety analysis of pollen derived from oilseed rape Ms8 × Rf3 in food or as food (EFSA, 2012). In this report, EFSA concluded that, considering the data available, no indication of potential concerns over the safety of the newly expressed phosphinothricin acetyl transferase (PAT) protein, barnase and barstar proteins, nor the occurrence of unintended effects in oilseed rape Ms8 × Rf3 pollen, have been identified that could raise safety concerns (EFSA, 2012).

In oilseed rape Ms8, the genes *bar* and *barnase* are introduced conferring tolerance to the herbicidal active ingredient glufosinate-ammonium, and male sterility, respectively. Oilseed rape Rf3 is also tolerant to glufosinate-ammonium, and expresses a restorer of fertility as a consequence of the introduced genes *bar* and *barstar*, respectively.

Molecular analysis has confirmed that the Ms8 and Rf3 inserts are present and that their structures are retained in oilseed rape Ms8 × Rf3. Result of the bioinformatic analyses of the flanking sequences and the open reading frames spanning the insert–genomic DNA junctions did not reveal safety issues. The levels of the PAT protein in oilseed rape Ms8 × Rf3 were similar to those of the single oilseed rape events Ms8 and Rf3.


[4] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. Official Journal of the European Communities, L268, 1–23.

The comparative analysis indicated that no biologically relevant differences were identified in the compositional, agronomic and phenotypic characteristics of oilseed rape Ms8 × Rf3 compared with its non-GM comparator oilseed rape, except for the newly expressed PAT, barnase and barstar proteins.

The safety of the newly expressed proteins present in oilseed rape Ms8, Rf3 and Ms8 × Rf3 were previously assessed by the EFSA GMO Panel in 2005 and 2009, and no safety concerns were identified for humans or animals, in the context of their intended uses. A 42-day broiler feeding study confirmed that oilseed rape Ms8 × Rf3 was as nutritious as its non-GM comparator. The new information provided in the present application does not raise concerns regarding toxicity and allergenicity of oilseed rape Ms8, Rf3 and Ms8 × Rf3. Thus, the EFSA GMO Panel reiterates its previous conclusion that oilseed rape Ms8, Rf3 and Ms8 × Rf3 are unlikely to have an adverse effect on human and animal health, in the context of their intended uses.

As this application does not cover cultivation of oilseed rape Ms8, Rf3 and Ms8 × Rf3, there is no requirement for scientific information on the possible environmental effects associated with the cultivation of oilseed rape Ms8, Rf3 and Ms8 × Rf3. In the event of the accidental release into the environment of viable oilseed rape Ms8, Rf3 and Ms8 × Rf3 seeds unintentionally present in food, there are no indications of an increased likelihood of establishment and spread of feral oilseed rape Ms8, Rf3 and Ms8 × Rf3, unless exposed to glufosinate-ammonium-containing herbicides. Likewise, evidence indicates that hybridising wild relatives that may theoretically have acquired the herbicide tolerance trait through vertical gene flow are neither more likely to establish nor to spread than their non-GM comparators in the absence of glufosinate-ammonium-containing herbicides. Considering the intended uses of oilseed rape Ms8, Rf3 and Ms8 × Rf3, potential interactions of feral oilseed rape Ms8, Rf3 and Ms8 × Rf3 plants with the biotic and abiotic environment are not considered to be an issue due to the low levels of exposure. Due to the intended uses of oilseed rape Ms8, Rf3 and Ms8 × Rf3, only a low level exposure of bacteria in the environment, including those in the gastrointestinal tract, to recombinant DNA from oilseed rape Ms8, Rf3 and Ms8 × Rf3 is expected. The unlikely but theoretically possible transfer of the recombinant genes from oilseed rape Ms8, Rf3 and Ms8 × Rf3 to bacteria does not raise concerns due to the lack of a selective advantage that would be provided to the recipients in the receiving environments. Additionally, tolerance and resistance to glufosinate-ammonium is widespread among bacteria in the environment making it unlikely that horizontal gene transfer would add to this natural background. The scope of the PMEM plan provided by the applicant is in line with the intended uses of oilseed rape Ms8, Rf3 and Ms8 × Rf3. Furthermore, the EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in its general surveillance plan.

In conclusion, the EFSA GMO Panel considers that the information available for oilseed rape Ms8, Rf3 and Ms8 × Rf3 addresses the scientific issues described in its relevant guidance documents and the scientific comments raised by the Member States, and that oilseed rape Ms8, Rf3 and Ms8 × Rf3 are unlikely to have an adverse effect on human and animal health or on the environment, in the context of their intended uses.

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## BACKGROUND

On 23 June 2010, the European Food Safety Authority (EFSA) received from the Competent Authority of Belgium an application (EFSA-GMO-BE-2010-81) for authorisation of the genetically modified (GM) herbicide tolerant oilseed rape Ms8 (Unique Identifier ACS-BNØØ5-8), Rf3 (Unique Identifier ACS-BNØØ3-6) and Ms8 × Rf3 (Unique Identifier ACS-BNØØ5-8 × ACS-BNØØ3-6) submitted by Bayer within the framework of Regulation (EC) No 1829/2003. The scope of this application covers food containing or consisting of, and food produced from or containing ingredients produced from oilseed rape Ms8, Rf3 and Ms8 × Rf3 (with the exception of processed oil) and excludes cultivation.

The EFSA GMO Panel has previously issued Scientific Opinions on oilseed rape Ms8, Rf3 and Ms8 × Rf3 related to: (i) the notification C/BE/96/01 for the placing on the market of glufosinate-tolerant hybrid oilseed rape Ms8 × Rf3 derived from GM parental lines Ms8 and Rf3 for import and processing for feed and industrial uses under Part C of Directive 2001/18/EC (EFSA, 2005a); and (ii) the renewal of the authorisation for continued marketing of existing (a) food and food ingredients produced from oilseed rape Ms8, Rf3 and Ms8 × Rf3, and (b) feed materials produced from oilseed rape Ms8, Rf3 and Ms8 × Rf3, under Regulation (EC) No 1829/2003 (EFSA, 2009a). In these Scientific Opinions, the EFSA GMO Panel concluded that oilseed rape Ms8, Rf3 and Ms8 × Rf3 are unlikely to have an adverse effect on human or animal health or, in the context of their proposed uses, on the environment. In addition, EFSA recently published a technical report on a safety analysis of pollen derived from oilseed rape Ms8 × Rf3 in food or as food (EFSA, 2012). In this report, EFSA concluded that considering the data available, no indication of potential concerns over the safety of the newly expressed PAT, barnase and barstar proteins, nor the occurrence of unintended effects in oilseed rape Ms8 × Rf3 pollen have been identified that could raise safety concerns (EFSA, 2012).

After receiving the application EFSA-GMO-BE-2010-81 and in accordance with Articles 5(2)(b) and 17(2)(b) of Regulation (EC) No 1829/2003, EFSA informed both Member States and the European Commission (EC), and made the summary of the application publicly available on the EFSA website.[5] 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 16 September 2011, EFSA received additional information requested under completeness check (requested on 3 August 2010). On 5 October 2011, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the EC, and consulted nominated risk assessment bodies of the Member States, including the national Competent Authorities within the meaning of Directive 2001/18/EC,[6] following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Member States had three months after the date of receipt of the valid application (until 5 January 2012) within which to make their opinion known.

The EFSA GMO Panel carried out a scientific risk assessment of oilseed rape Ms8, Rf3 and Ms8 × Rf3 for food containing or consisting of, and food produced from or containing ingredients produced from these GM plants (with the exception of processed oil), in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. When carrying out the safety evaluation, the EFSA GMO Panel took into account the appropriate principles described in its guidance documents for the risk assessment of GM plants and derived food and feed (EFSA 2006a), for the risk assessment of GM plants containing stacked transformation events (EFSA, 2007a) and on the post-market environmental monitoring (PMEM) of GM plants (EFSA, 2006b, 2011a). Furthermore, the scientific comments of

[5] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2010-00947

[6] 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. Official Journal of the European Communities L106, 1–38.

the Member States, the additional information provided by the applicant, relevant scientific publications and information from previous applications on oilseed rape Ms8, Rf3 and Ms8 × Rf3 were taken into account.

On 29 November 2011, 25 January 2012 and 24 May 2012, the EFSA GMO Panel requested additional information from the applicant. The applicant provided the requested information on 3 April 2012 and 29 May 2012.

In giving its opinion on application EFSA-GMO-BE-2010-81 to the EC, 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 six months from the acknowledgement of the valid application. As additional information was requested by the EFSA GMO Panel, the time limit of six 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).

## TERMS OF REFERENCE

The EFSA GMO Panel was requested to carry out a risk assessment of oilseed rape Ms8, Rf3 and Ms8 × Rf3 with the scope for food containing or consisting of, and food produced from or containing ingredients produced from oilseed rape Ms8, Rf3 and Ms8 × Rf3 (with the exception of processed oil) in accordance with Article 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 requirements based on the outcome of the risk assessment and, in the case of food 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 EFSA GMO Panel was not requested to give a Scientific Opinion on information required under Annex II to the Cartagena Protocol. Furthermore, the EFSA GMO Panel did also not consider proposals for labelling and 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.

# ASSESSMENT

## 1. INTRODUCTION

The genetically modified (GM) oilseed rape Ms8 (Unique Identifier ACS-BNØØ5-8), Rf3 (Unique Identifier ACS-BNØØ3-6) and Ms8 × Rf3 (Unique Identifier ACS-BNØØ5-8 × ACS-BNØØ3-6) were evaluated with reference to their intended uses, taking account of the appropriate principles described in the relevant guidance document of the EFSA GMO Panel for the risk assessment of GM plants and derived food and feed (EFSA, 2006a), for the risk assessment of GM plants containing stacked transformation events (EFSA, 2007a) and on the post-market environmental monitoring (PMEM) of GM plants (EFSA, 2011a). The evaluation of the risk assessment presented here is based on the information provided in the application, as well as additional information obtained from the applicant, scientific comments submitted by the Member States and relevant scientific publications. Further information from previous applications for placing on the European Union market of oilseed rape Ms8, Rf3 and Ms8 × Rf3 were taken into account (EFSA, 2005a, 2009a).

The male sterile (Ms8) and fertility restorer (Rf3) parental lines of oilseed rape Ms8 × Rf3 form an $F_1$ hybrid system, which ensures a high proportion of hybrid seed and, as a consequence, higher yields as a result of heterosis. Oilseed rape Ms8 was developed to express a *barnase* gene (encoding the ribonuclease protein, barnase) in the tapetum cells of its anthers, which results in death of the tapetum cells and male-sterile plants arising from the production of non-viable pollen. Oilseed rape Rf3 was developed to express a *barstar* gene in the tapetum cells, which encodes a barstar protein to prevent the functioning of barnase. Therefore, in the stack Ms8 × Rf3, the barstar protein from Rf3 inhibits barnase from Ms8 resulting in viable pollen and restored fertility and seed production capability in the hybrid. Both Ms8 and Rf3 also express the phosphinothricin acetyl transferase (PAT) protein, encoded by the *bar* gene, conferring tolerance to the herbicidal active ingredient glufosinate-ammonium.

## 2. ISSUES RAISED BY MEMBER STATES

The issues raised by the Member States are addressed in Annex G of the EFSA overall opinion[7] and have been considered throughout this scientific opinion.

## 3. MOLECULAR CHARACTERISATION

### 3.1. Evaluation of relevant scientific data

#### *3.1.1. Characterisation of the single events*[8]

3.1.1.1. Ms8 (male sterile line)

Oilseed rape Ms8 was developed through *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*)-mediated transformation by the insertion of two expression cassettes: (i) the *barnase* cassette includes a tapetum-specific promoter (*Pta29*, which restricts expression of the barnase ribonuclease to the tapetum cells during anther development); and (ii) the *bar* expression cassette in which the *PssuAt* promoter (active in all green tissues) controls the expression of the *bar* gene encoding the PAT protein conferring tolerance to glufosinate-ammonium-containing herbicides.

Molecular characterisation data have established that Ms8 contains a single copy of the transfer DNA (T-DNA) at a single locus and that vector backbone sequences are absent. Polymerase chain reaction (PCR) analyses have confirmed that the flanking regions of the insert are of oilseed rape genomic DNA origin. The pre-insertion locus was preserved, except for the deletion of 19 bp and the addition of 3 bp. Updated bioinformatic analyses of the 5′ and 3′ flanking regions did not reveal disruption of

[7] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2010-00947
[8] Technical dossier / Section D2.

known endogenous oilseed rape genes, or the creation of open reading frames that would raise a safety issue.

Southern blot analysis of oilseed rape Ms8 and maintenance of the phenotype indicated genetic and phenotypic stability of the event over multiple generations (EFSA, 2005a).

3.1.1.2. Rf3 (fertility restorer line)

Oilseed rape Rf3 was developed through *Agrobacterium*-mediated transformation by the insertion of two expression cassettes present in plasmid pTHW118: (i) the *barstar* cassette includes a tapetum-specific promoter (*Pta29*) to restrict expression of barstar ribonuclease inhibitor to the tapetum cells; and (ii) the *bar* expression cassette in which the *PssuAt* promoter (active in all green tissues) controls the expression of the *bar* gene encoding the PAT protein conferring tolerance to glufosinate-ammonium-containing herbicides.

Molecular characterisation data have established that Rf3 contains one partial copy of the T-DNA, in which only part of the *Pta29* promoter was inserted, flanked by another partial copy in an inverted orientation, which includes a complete *barstar* gene cassette (*Pta29* promoter, *barstar* coding region, *nos* terminator) and a part of the *PssuAt* promoter. Vector backbone sequences are absent from Rf3. PCR analyses have confirmed that the flanking regions of the insert are of oilseed rape genomic DNA origin. Analysis of the insertion locus revealed that 815 bp flanking the 5′ of the insert was duplicated at the 3′ flank and that 51 bp were lost from the pre-insertion locus. At the right border junction, 5-bp filler DNA is present when compared with the wild-type DNA. Updated bioinformatic analyses of the 5′ and 3′ flanking regions did not reveal disruption of known endogenous oilseed rape genes or the creation of open reading frames that would raise a safety issue.

Southern blot analysis of oilseed rape Rf3 and maintenance of the phenotype indicated genetic and phenotypic stability of the event over multiple generations (EFSA, 2005a).

### *3.1.2. Method of production of oilseed rape Ms8 × Rf3*[9]

Conventional breeding methods were used to develop oilseed rape Ms8 × Rf3 and no new genetic modification was involved. The inserts present in oilseed rape Ms8 × Rf3 were derived from oilseed rape lines containing the single events Ms8 and Rf3. Both of these oilseed rape events have been the subject of earlier scientific opinions of the EFSA GMO Panel (EFSA, 2005a, 2009a). Oilseed rape Ms8 × Rf3 combines a male sterility-based hybridisation system with tolerance to glufosinate-ammonium-containing herbicides.

### *3.1.3. Transgene constructs in Ms8 × Rf3*[10]

The integrity of the individual inserts present in oilseed rape Ms8 × Rf3 was investigated using Southern blot analyses in an oilseed rape stack produced by conventional crossing (oilseed rape Ms8 × Rf3 × GT73).[11] This involved the use of DNA probes specific for the single inserts and enzymatic digestions informative of the structure of both events, including the junction regions with the host genomic DNA. The predicted DNA hybridisation patterns of both single events were retained in oilseed rape Ms8 × Rf3 × GT73. Based on this result and on the maintenance of the phenotype over several generations, it can be concluded that the integrity of the inserts is maintained in oilseed rape Ms8 × Rf3.

[9] Technical dossier / Section C1.
[10] Technical dossier / Section D2.
[11] Additional information, April 2012.

#### *3.1.4. Information on the expression of the insert*[12]

The levels of the *barnase*, *barstar* and *bar* gene transcripts and the corresponding proteins were analysed by northern and western blotting, enzyme-linked immunosorbent assay (ELISA) and strip tests in oilseed rape Ms8, Rf3 and Ms8 × Rf3.

The spatial and temporal expression of *barnase* and *barstar* genes is restricted to the flower buds at 0.2–0.4 and 3.2–6.4 ng/g total RNA, respectively.[13]

The PAT protein is expressed in various tissues with a higher level in green parts and only trace amounts in other tissues.[14] The PAT protein expression was detected in pollen, but the level was not quantified. However, as the PAT messenger RNA was not detected in pollen by northern blot analysis, it could be inferred that the level of the PAT protein in pollen is likely to be very low. For the expression analysis in seeds, samples of spring oilseed rape were collected from a field trial conducted in Belgium during 2000. The plants were treated with a glufosinate-ammonium-containing herbicide. Levels of the PAT protein in seed were 0.07–0.10 µg/g fresh weight (fw), 0.15–0.31 µg/g fw and 0.11–0.22 µg/g fw for Ms8, Rf3 and Ms8 × Rf3, respectively.[15]

#### *3.1.5. Inheritance and stability of inserted DNA*[16]

The stability of the inserted DNA in oilseed rape Ms8 and Rf3 was demonstrated previously (EFSA, 2005a). The Southern blot analysis data show that the integrity of the inserts present in the single events is retained in Ms8 × Rf3. This is supported by Southern blot analyses on oilseed rape Ms8 × Rf3 × GT73.[17]

### 3.2. Conclusion

Oilseed rape Ms8 × Rf3 is produced by conventional crossing. Southern blot analyses demonstrated that the structures of the inserts in oilseed rape Ms8 and Rf3 were retained in oilseed rape Ms8 × Rf3. Updated bioinformatic analyses of the flanking sequences and the open reading frames spanning the insert–plant DNA junctions did not indicate any safety issues. The levels of the PAT protein in oilseed rape Ms8, Rf3 and Ms8 × Rf3 have been sufficiently analysed. Molecular characterisation data do not indicate safety issues arising from combining the single events Ms8 and Rf3 to produce oilseed rape Ms8 × Rf3.

The EFSA GMO Panel concludes that the molecular characterisation of oilseed rape Ms8, Rf3 and Ms8 × Rf3 does not raise safety issues.

## 4. COMPARATIVE ANALYSIS

### 4.1. Evaluation of relevant scientific data

Unless specifically indicated, the information provided in this application and described in the following sections has been evaluated previously by the EFSA GMO Panel (EFSA, 2005a).

#### *4.1.1 Choice of comparator and production of material for the compositional assessment*

In its previous scientific opinion of 2005, the EFSA GMO Panel evaluated compositional data, which were obtained by analysis of materials from field trials performed at 12 different locations in Belgium during the seasons 2000–2001 and 2001–2002. The non-GM comparator used in the comparative analysis of oilseed rape Ms8 × Rf3 was the open-pollinated winter oilseed rape line PP0005B. Both

[12] Technical dossier / Section D3.
[13] EFSA-GMO-RX-Ms8Rf3, Technical dossier / Section D3, Tables 16–19.
[14] Technical dossier / Section D3.a.
[15] EFSA-GMO-RX-Ms8Rf3, Technical dossier / Section D3, Table 20.
[16] Technical dossier / Section D5.
[17] Additional information, April 2012.

the Ms8 and Rf3 events were originally generated in a spring oilseed rape (*Brassica napus*, variety Drakkar), but backcrossed into the winter oilseed rape line PP0005B, using conventional backcrossing techniques to produce a comparable genetic background for the hybrid and the comparator. Oilseed rape Ms8 × Rf3 used in the field trials resulted from crossing plants from the Ms8 event that were backcrossed to the PP0005B line seven times and plants from the Rf3 event that were backcrossed five times and then subjected to three selfings to produce a homozygous Rf3/Rf3 PP0005B parental line. Seeds from glufosinate-ammonium-treated and -untreated oilseed rape Ms8 × Rf3 and the non-GM comparator were harvested for compositional analysis. The same field trials were used to assess the agronomic performance of oilseed rape Ms8 × Rf3 (EFSA, 2005a).

In addition to data from these previously evaluated materials, the applicant provided, in the frame of the current application, compositional data from additional field trials that had been performed at five locations in Canada during the 2008 growing season. In these field trials, oilseed rape Ms8 and Rf3 treated with the target herbicide were compared with comparators consisting of negative segregants from these oilseed rape events untreated with the target herbicide. Although the EFSA GMO Panel is of the opinion that the risk assessment of a GMO based exclusively on the comparison with a negative segregant is not sufficient to perform a proper safety evaluation, these data may provide supplementary information in addition to the data previously assessed in 2005.

### *4.1.2 Compositional analysis*[18]

The EFSA GMO Panel previously evaluated the comparative assessment of compositional data of oilseed rape Ms8 × Rf3 (treated either with glufosinate-ammonium-containing herbicide or with conventional herbicides) and a non-GM comparator treated with conventional herbicides grown in Belgian field trials during two seasons (EFSA, 2005a).

Harvested material from field trials performed in 12 different locations in Belgium during the seasons 2000–2001 and 2001–2002 were used for compositional analysis (EFSA, 2005a).

For compositional analysis at each location, seeds were harvested from 12 plots providing four samples each for conventionally treated oilseed rape Ms8 × Rf3, glufosinate-ammonium-treated oilseed rape Ms8 × Rf3 and the conventionally treated non-GM comparator, amounting to 144 samples in total for all locations. These samples were analysed on key nutrients, anti-nutrients and toxicants (OECD, 2001), including proximates, micronutrients, such as minerals and tocopherols, anti-nutrients as phytic acid and glucosinolates, and total spectrum of amino acids and fatty acids (the latter including erucic acid). The statistical analysis performed by the applicant did not indicate that unintended effects had occurred as a result of the genetic modification. An analysis of variance (ANOVA) statistical analysis of glucosinolate data, as provided by the applicant, showed some statistically significant differences in the contents of alkenyl glucosinolates and total glucosinolates between the GM oilseed rape and its non-GM comparator. However, these differences were not considered biologically relevant (EFSA, 2011b) given the reported natural variations in these compounds in oilseed rape (OECD, 2001). The absolute differences in glucosinolate levels between the GM and non-GM oilseed rape seed samples amounted an increase up to 4 µmol/g on a mean total glucosinolate level in GM seed not exceeding 16 µmol/g (EFSA, 2005a). This level was below the threshold glucosinolate content of 25 µmol/g, set by the European Commission for certified seed of "double zero" varieties listed in the Common Catalogue of Varieties of Agricultural Plant Species (EC, 1999).

The EFSA GMO Panel is of the opinion that the extensive comparative compositional analysis provides no indication for unintended effects of the genetic modification that would raise safety concerns.

[18] Technical dossier / Sections D.7.1., D.7.2., D.7.3.

Compositional data not previously assessed by the EFSA GMO Panel were obtained by analysis of oilseed rape harvested from field trials performed in Canada in 2008. In these field trials, oilseed rape Ms8 and Rf3 treated with the target herbicide were compared with comparators consisting of negative segregants from these oilseed rape events untreated with the target herbicide. The use of negative segregants as sole comparators is not in line with EFSA guidance documents (EFSA, 2006a, 2007a). The EFSA GMO Panel considers that the additional information provided is supplementary to the data already assessed, and that the outcomes do not lead the Panel to change its previous conclusion on the compositional characteristics.

#### *4.1.3 Agronomic traits and GM phenotype*[19]

The agronomic and phenotypic data on oilseed rape Ms8 × Rf3 have been previously evaluated by the EFSA GMO Panel (EFSA, 2005a).

At each location, the agronomic performance of oilseed rape Ms8 × Rf3, half of them treated according to conventional herbicides regimes and the other half treated with glufosinate-ammonium-containing herbicides, was compared with the non-GM comparator and a local oilseed rape variety, both treated according to conventional herbicide regimes. The agronomic performance was monitored from germination until harvest for a number of key agronomic parameters, such as establishment, vigour, flowering, height, maturity, lodging and yield (EFSA, 2005a).

The agronomic performance of oilseed rape Ms8 × Rf3 was not affected by the genetic modification, except for slightly higher yield due to hybrid vigour (EFSA, 2005a).

### 4.2. Conclusion

Based on the information available, the EFSA GMO Panel concludes that no biologically relevant differences were identified in the composition, agronomic and phenotypic characteristics of oilseed rape Ms8 × Rf3, as compared with its non-GM comparator, except for the newly expressed PAT, barnase and barstar proteins.

## 5. FOOD/FEED SAFETY ASSESSMENT

### 5.1. Evaluation of relevant scientific data

Unless specifically indicated, the information provided in this application and described in the following sections has been evaluated previously by the EFSA GMO Panel (EFSA, 2005a, 2009a).

#### *5.1.1. Product description and intended use*[20]

The scope of application EFSA-GMO-BE-2010-81 is for food containing or consisting of, and food produced from or containing ingredients produced from, oilseed rape Ms8, Rf3 and Ms8 × Rf3 (with the exception of processed oil).

The applicant indicated that the scope of the current application (EFSA-GMO-BE-2010-81) is to complement existing authorised uses of oilseed rape Ms8, Rf3 and Ms8 × Rf3, including the accidental unintentional presence of traces of oilseed rape Ms8, Rf3 and Ms8 × Rf3 seeds in food.

In its previous scientific opinion, the EFSA GMO Panel noted that only seeds of oilseed rape are used in the human food and animal feed chain. In the human diet, oilseed rape is only used after being processed into food grade vegetable oil. The only oilseed rape product for human use is the refined oil, which has already been notified within the EU.[21] The main by-product from oil processing, the

[19] Technical dossier / Section D.7.4.
[20] Technical dossier / Section D.7.5., D.7.7.
[21] http://europa.eu.int/comm/food/dyna/gm_register/index_en.cfm

mechanically and/or solvent-extracted meal, is used as a protein-rich feed for all classes of livestock (EFSA, 2005a).

In the present application as additional information,[22] the applicant provided a dietary intake assessment of pollen from oilseed rape Ms8, Rf3 and Ms8 × Rf3, which resulted in a low level of exposure. In addition, EFSA recently published a technical report on a safety analysis of pollen derived from oilseed rape Ms8 × Rf3 in food or as food (EFSA, 2012). In this report, EFSA concluded that, considering the data available, no indication of potential concerns over the safety of the newly expressed PAT, barnase and barstar proteins, nor the occurrence of unintended effects in oilseed rape Ms8 × Rf3 pollen have been identified that could raise safety concerns (EFSA, 2012).

The genetic modification results in the expression of the PAT, barstar and barnase proteins in oilseed rape Ms8 × Rf3. PAT protein confers to the plants tolerance to the herbicidal active ingredient glufosinate-ammonium. Thus, the modification is intended to improve agronomic performance only and is not intended to influence the nutritional properties, the processing characteristics or the overall use of oilseed rape as a crop. Oilseed rape Ms8, Rf3 and Ms8 × Rf3 is intended to be processed in the same way as any conventional oilseed rape.

The PAT protein is expressed in various tissues with a higher level in green parts and only trace amount in other tissues (see section 3.1.4). Barnase and barstar proteins or the barnase–barstar complex are not detected in plant tissues outside the flower buds, and therefore are not detected in seeds, pollen and unprocessed meal.

### *5.1.2. Effects of processing*[23]

Based on the data obtained in the comparative compositional analysis of oilseed rape Ms8 × Rf3 and its non-GM comparator (section 4.1.2), the EFSA GMO Panel considers that there are no reasons to assume that the effect of processing on products derived from oilseed rape Ms8, Rf3 and Ms8 × Rf3 would be different from that on products from conventional oilseed rape.

### *5.1.3. Toxicological assessment*[24]

#### 5.1.3.1. Protein used for safety assessment

Of the novel proteins expressed in oilseed rape Ms8, Rf3 and Ms8 × Rf3, only the PAT protein can be expected to be present in the food chain. As the expression level of the PAT protein in the GM oilseed rape is very low, purification of the protein in sufficient quantity from the GM plant would be difficult. Therefore, the safety studies were conducted with a PAT protein produced in *Escherichia coli*. Extensive examination of the nature of the plant and bacterial PAT proteins have shown a high degree of similarity, based on their size and sequence homology, enzymatic activity, immunoreactivity and absence of glycosylation (Herouet et al., 2005). The EFSA GMO Panel accepted the test material derived from *E. coli* for the safety assessment of the PAT protein in oilseed rape (EFSA, 2005a).

#### 5.1.3.2. Toxicological assessment of newly expressed proteins

Using ELISA, the PAT protein was detected in seeds. The PAT protein expression was also revealed in pollen, and, although the level was not quantified, it is likely to be very low (see section 3.1.4.). Barnase and barstar proteins are only expressed in the tapetum cells of the flower buds and therefore will not occur in food derived from oilseed rape Ms8 × Rf3 seeds or pollen. In this context, the results of western blot analyses indicated that barnase and barstar are not detected in pollen.[25]

(a) Bioinformatics analysis

[22] Additional information received in March and May 2012.
[23] Techical dossier / Section D.7.6.
[24] Technical dossier / Section D.7.8.
[25] Technical dossier EFSA-GMO-RX-Ms8 × Rf3.

A bioinformatics study, which was previously evaluated by the EFSA GMO Panel, showed no relevant similarity between the newly expressed proteins PAT, barnase, barstar, and known toxic proteins (EFSA, 2009a).

In this application, the updated bioinformatics studies provided by the applicant confirmed the results of the previous studies.

(b) Degradation in simulated digestive fluids

*In vitro* digestibility studies have shown rapid degradability of the PAT protein. In a pepsin digestion assay, the PAT protein was digested within 30 seconds of incubation in the presence of pepsin at pH 2.0. In a pancreatin digestion assay, the PAT protein was digested into smaller fragments within seconds, whereas the complete degradation of a 7-kDa fragment was achieved within 5 minutes of incubation in the presence of pancreatin at pH 7.5 (EFSA, 2005a).

(c) Animal toxicity testing

Oral toxicity studies with the PAT protein encoded by the *bar* gene are not available, but a 14-day repeated dose oral toxicity study conducted in rats fed with the PAT protein encoded by the *pat* gene up to dietary levels of 50,000 ppm did not induce toxic effects. The PAT/pat and PAT/bar proteins have been shown to be structurally and functionally equivalent (Wehrmann et al., 1996; Herouet et al., 2005). Therefore, the EFSA GMO Panel accepted the data from the study with PAT/pat protein, in order to assess the safety of the PAT/bar protein (EFSA, 2005a).

The EFSA GMO Panel has previously evaluated the safety of the PAT protein in the context of several applications for the placing on the EU market of GM crops expressing PAT, and no safety concerns were identified (e.g., EFSA, 2005b, 2006c, 2007b,c,d, 2008).

5.1.3.3. Toxicological assessment of new constituents other than proteins

No new constituents other than the novel proteins are expressed in oilseed rape Ms8, Rf3 and Ms8 × Rf3, and no biologically relevant changes in the composition of oilseed rape Ms8 × Rf3 were detected in the comparative compositional analysis (section 4.1.2). Therefore, a toxicological assessment of new constituents is not applicable.

5.1.3.4. Toxicological assessment of the whole GM food/feed

The EFSA GMO Panel previously concluded that oilseed rape Ms8, Rf3 and Ms8 × Rf3 are unlikely to have an adverse effect on human and animal health (EFSA, 2005a, 2009a).

A molecular characterisation undertaken showed that the stability of the transgene inserts in oilseed rape Ms8 and Rf3 was demonstrated over multiple generations, implying that the integrity of the inserts was maintained throughout microsporogenesis and pollen production (EFSA, 2005a). At the request of the EFSA GMO Panel, the applicant provided additional information demonstrating the integrity of the individual inserts present in oilseed rape Ms8 × Rf3 when these were brought together by crossing (see section 3.1.5). As no biologically relevant differences were identified in the compositional characteristics of oilseed rape Ms8 × Rf3 in comparison with its non-GM comparator, except for expressing the barnase, barstar and PAT proteins, the EFSA GMO Panel is of the opinion that no additional animal safety studies are required.

### *5.1.4. Allergenicity assessment*[26]

The strategies used when assessing the potential allergenic risk focus on the characterisation of the source of the recombinant protein, the potential of the newly expressed protein to induce sensitisation

[26] Technical dossier / Section D.7.9.

or to elicit allergic reactions in already sensitised persons, and whether the transformation may have altered the allergenic properties of the modified food.

5.1.4.1. Assessment of allergenicity of the newly expressed proteins

A weight-of-evidence approach is recommended when assessing the potential allergenicity of a newly expressed protein, taking into account all of the information obtained with various test methods, as no single experimental method yields decisive evidence for allergenicity (Codex Alimentarius, 2009; EFSA, 2010).

The PAT protein is the only newly expressed protein present in oilseed rape Ms8, Rf3 and Ms8 × Rf3 seed and pollen. Barnase and barstar proteins are expressed only in the tapetum cells of the flower buds and therefore will not occur in food or feed derived from oilseed rape Ms8, Rf3 and Ms8 × Rf3 seed or pollen.

Bioinformatic studies previously evaluated by the EFSA GMO Panel revealed no relevant similarity between the newly expressed proteins PAT, barnase and barstar and known allergens (EFSA, 2009a).

In this application, updated bioinformatics studies were provided. Analysis of the amino acid sequences of the newly expressed proteins revealed no relevant similarity between barnase, barstar and PAT proteins and known allergens and thus confirmed the results of the previous study.

The *in vitro* digestibility studies showed that the PAT protein was rapidly degraded (see section 5.1.3.2.).

Additionally, the EFSA GMO Panel previously evaluated the allergenicity of the PAT protein in the context of other applications for the placing on the EU market of GM crops expressing PAT and considered it unlikely to be allergenic (e.g., EFSA, 2005b, 2006c, 2007b,c,d, 2008).

The EFSA GMO Panel concludes that there are no indications that these newly expressed proteins in oilseed rape Ms8, Rf3 and Ms8 × Rf3 are allergenic.

5.1.4.2. Assessment of allergenicity of the whole GM plant

According to EFSA guidance documents (EFSA, 2006a, 2011c), when the recipient GM plant of the introduced gene is known to be allergenic, the applicant should test any potential change in the allergenicity of the whole GM plant by comparing the allergen repertoire with that of its appropriate comparator(s). In this context, oilseed rape is not considered a common allergenic food (EC, 2007), although rare cases of occupational allergy to inhaled dust/flour and sensitisation and allergic reactions in atopic patients have been reported (Monsalve et al., 1997; Suh et al., 1998; Poikonen et al., 2006; Puumalainen et al., 2006).

Additionally, the allergenicity of the whole GM plant can theoretically be increased by unintended changes at the insertion sites by modifying the expression of endogenous genes (potential allergens) or by producing new allergens. However, bioinformatics analyses of the DNA sequence at the insertion sites did not indicate: (i) changes in the expression of endogenous genes; or (ii) creation of open reading frames at the insert–plant DNA junctions that are likely to be translated into allergenic peptides.

In the context of the present application, and considering all information available, there is no evidence that the genetic modification might alter the pattern of expression of endogenous proteins (potential allergens) in the oilseed rape Ms8, Rf3 and Ms8 × Rf3 and thereby significantly change the overall allergenicity of the whole GM plant.

### *5.1.5. Nutritional assessment*[27]

A 42-day feeding study was carried out on male broiler chickens (420 Ross chickens). Animals were divided in three groups (140 chickens per group) and were fed diets containing 10 % GM oilseed rape Ms8 × Rf3 that was either treated with the target herbicides or untreated, or were fed 10 % of a commercial non-GM oilseed rape variety with a genetic background similar to oilseed rape Ms8 × Rf3. In all cases, full-fat hammer-milled rapeseed was used. No significant differences in any of the parameters studied (animal health, survival, feed intake, weight gain, feed conversion and carcass and muscle weights) were noted between the groups fed diets containing the oilseed rape Ms8 × Rf3 and the non-GM comparator (EFSA, 2009a).

The broiler feeding study supports the results of the comparative compositional analysis and confirms that oilseed rape Ms8 × Rf3 is as nutritious as a commercial non-GM oilseed rape with a genetic background similar to oilseed rape Ms8 × Rf3 (EFSA, 2009a).

### *5.1.6. Post-market monitoring*[28]

No biologically relevant compositional, agronomic or phenotypic changes were identified in oilseed rape Ms8 × Rf3, as compared with its non-GM comparator, with the exception of the newly expressed proteins. Furthermore, the overall intake or exposure is not expected to change due to the introduction of oilseed rape Ms8, Rf3 and Ms8 × Rf3 into the market. Therefore, and in line with its guidance document for the risk assessment of GM plants and derived food and feed (EFSA, 2006), the EFSA GMO Panel considers that post-market monitoring of the food/feed derived from oilseed rape Ms8, Rf3 and Ms8 × Rf3 is not necessary.

## 5.2. Conclusion

From the data on the expression of novel proteins, the PAT protein is the only newly expressed protein in the seeds and pollen of oilseed rape Ms8, Rf3 and Ms8 × Rf3. Barnase and barstar are expressed only 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 (section 3.1.4). The *in vitro* digestibility studies showed that the PAT protein was rapidly degraded. The EFSA GMO Panel has previously evaluated the safety of the PAT protein not only in oilseed rape (EFSA, 2005a, 2009a) but also in other crops and did not identify any safety issue regarding potential toxicity and allergenicity of the protein (section 5.1.3.2). The PAT protein expression was detected in pollen, and, although the level was not quantified, it is likely to be very low (see section 3.1.4). Additionally and also 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 from that in other parts of the plant. Therefore, the toxicity and allergenicity datasets assessed are also applicable to the newly expressed protein in pollen.

The applicant provided a dietary intake assessment of pollen from oilseed rape Ms8, Rf3 and Ms8 × Rf3 resulting in a low level of exposure. Additionally, EFSA recently concluded that, considering the data available, no indication of potential concerns over the safety of the newly expressed PAT, barnase and barstar proteins, or the occurrence of unintended effects in oilseed rape Ms8 × Rf3 pollen, have been identified that could raise safety concerns (EFSA, 2012).

A broiler feeding study confirmed that oilseed rape Ms8 × Rf3 is as nutritious as its non-GM comparator (EFSA, 2009a). This study supports the conclusions of the comparative analysis showing that there are no biologically relevant differences in the composition of oilseed rape Ms8 × Rf3 compared with a non-GM comparator oilseed rape, except for the newly expressed proteins.

[27] Technical dossier / Section D.7.10.
[28] Technical dossier / Section D.7.11.

A review of peer-reviewed scientific data[29] on oilseed rape Ms8, Rf3 and Ms8 × Rf3 and derived food and feed, relevant for the safety assessment, revealed that there was no new information that would require changes of previous EFSA GMO Panel scientific opinions on oilseed rape Ms8, Rf3 and Ms8 × Rf3.

In line with previous opinions (EFSA, 2005a, 2009a), the EFSA GMO Panel considers that oilseed rape Ms8, Rf3 and Ms8 × Rf3 are unlikely to have an adverse effect on human and animal health, in the context of their intended uses.

## 6. ENVIRONMENTAL RISK ASSESSMENT AND MONITORING PLAN

### 6.1. Evaluation of relevant scientific data

Oilseed rape Ms8 × Rf3 is a hybrid consisting of Ms8, with the *barnase* gene conferring male sterility caused by non-development of the tapetum into anthers and the *bar* gene conferring resistance to the herbicidal active ingredient glufosinate-ammonium, and Rf3, which contains the *barstar* gene which expresses a male fertility restorer and the *bar* gene. The scope of the application covers the use of oilseed rape Ms8, Rf3 and Ms8 × Rf3 as food containing or consisting of, and food produced from or containing ingredients produced from, the GM plant (including the accidental unintentional presence of viable seeds and pollen of oilseed rape Ms8 × Rf3 but excluding refined oil) and does not include cultivation. Considering the intended uses of oilseed rape Ms8, Rf3 and Ms8 × Rf3, the environmental risk assessment is concerned with the accidental release into the environment of viable seeds of oilseed rape Ms8, Rf3 and Ms8 × Rf3 unintentionally present in food, and with the horizontal gene transfer to bacteria occurring in the environment or human digestive tract. As the scope of the present application excludes cultivation, environmental concerns in the EU pertaining to the use of glufosinate-ammonium-containing herbicides on oilseed rape Ms8, Rf3 and Ms8 × Rf3 do not apply.

#### *6.1.1. Environmental risk assessment*

6.1.1.1. Effects on plant fitness due to the genetic modification[30]

Agronomic data on oilseed rape Ms8 × Rf3 have indicated that it is a high-yielding hybrid with the ability to survive treatment with glufosinate-ammonium-containing herbicides but that the greater than average vigour and productivity are within the range found in commercial oilseed varieties, some of which are $F_1$ hybrids. Oilseed rape Ms8 is male sterile, and therefore is an obligate outcrosser that is more likely to produce $F_1$ hybrid seeds with other oilseed rape varieties and compatible wild relatives. Both oilseed rape Ms8 and Rf3 would also tolerate glufosinate-ammonium-containing herbicides.

In its 2005 scientific opinion, the EFSA GMO Panel concluded, based on a comparative analysis of agronomic traits and composition of oilseed rape Ms8 × Rf3, that "there was no indication of unintended effects of the genetic modification", and that "Ms8 × Rf3 oilseed rape was considered comparable with conventional oilseed rape, except for the expression of the new proteins". The EFSA GMO Panel also indicated that "Ms8 × Rf3 oilseed rape would generally not show any enhanced fitness and would behave as conventional oilseed rape" (EFSA, 2005a).

Demographic studies of feral oilseed rape have shown the ability of oilseed rape to establish self-perpetuating populations outside agricultural areas, mainly in semi-natural and ruderal habitats in different countries (reviewed by Devos et al., 2012). Oilseed rape is generally regarded as an opportunistic species and can take advantage of disturbed sites due to its potential to germinate and capture resources rapidly. Once established in competition-free germination sites, feral populations become extinct over a period of years. A 10-year survey (1993–2002) along road verges of a motorway revealed that most quadrats showed transient populations lasting 1–4 years (Crawley and

[29] Additional information received in March 2012.
[30] Technical dossier / Sections D4, D9.1 and D9.2.

Brown, 2004). These data and data from other demographic studies indicate a substantial turnover of populations of feral oilseed rape: only a small percentage of populations occur at the same location over successive years, whereas the majority appears to die out rapidly (Crawley and Brown, 1995, 2004; Charters et al., 1999; Peltzer et al., 2008; Elling et al., 2009; Knispel and McLachlan, 2009; Nishizawa et al., 2009; Squire et al., 2011). If habitats are disturbed on a regular basis by anthropogenic activities, such as mowing, herbicide applications or soil disturbance, or natural occurrences, such as flooding, then feral populations can persist for longer periods (Claessen et al., 2005a; Garnier et al., 2006). The persistence or recurrence of a population in one location is variously attributed to replenishment with fresh seed spills, recruitment from seed emerging from the soil seedbank or shed by resident feral adult plants, or redistribution of feral seed from one location to another. While many feral populations observed over multiple years were transient at a local scale (e.g. Crawley and Brown, 1995, 2004; Knispel et al., 2008), this apparent transience is likely to be counterbalanced on a landscape scale by repeated seed addition and redistribution from various sources (Pivard et al., 2008a,b). On a larger scale in the landscape, feral oilseed rape can thus be considered long lived, with a proportion of the populations founded by repeated fresh seed spills from both agricultural fields and transport and the remainder resulting from the continuous recruitment of seed from local feral soil seedbanks (Pivard et al., 2008a,b).

The above-mentioned demographic studies and surveys monitoring transgene presence in feral oilseed rape populations indicate that feral oilseed rape is generally confined to ruderal habitats and that GM herbicide-tolerant (GMHT) oilseed rape also behaves as a typical non-persistent ruderal plant. The ability of oilseed rape to successfully invade natural habitats is limited principally by the availability of seed germination sites and interspecific plant competition (Crawley et al., 1993, 2001; Crawley and Brown, 1995; Hails et al., 2006; Damgaard and Kjaer, 2009). Moreover, in controlled sowings into road verges, field margins and wasteland, very few seedlings survived to maturity due to grazing (e.g. by molluscs) and abiotic stress (Charters et al., 1999). Field studies (such as transplant or seed sowing experiments) have confirmed that herbicide tolerance traits in oilseed rape do not confer a fitness advantage, unless the specific herbicides for which tolerance is obtained are applied (Crawley et al., 1993, 2001). Crawley et al. (1993, 2001) have assessed the invasive potential of GM plants directly by releasing them into natural habitats and by monitoring their fitness in subsequent generation(s). GMHT oilseed rape introduced into 12 different habitats at three sites across the UK failed to persist in established vegetation: in none of the natural plant communities considered was oilseed rape found after 3 years even when vegetation had been removed in the first year of sowing (Crawley et al., 1993, 2001). These experiments demonstrated that the genetic modification per se does not enhance ecological fitness. Ecophysiological experiments on the comparative fitness of the GM plant and its non-GM counterpart and subsequent modelling did not indicate that genes conferring herbicide tolerance significantly alter the competitive ability of GM plants (Fredshavn et al., 1995; Warwick et al., 1999, 2003, 2009; Norris and Sweet, 2002; Claessen et al., 2005a,b; Garnier and Lecomte, 2006; Garnier et al., 2006; Simard et al., 2005; Londo et al., 2010). Beckie et al. (2004) showed that GMHT oilseed rape with single or multiple herbicide tolerance traits is not more persistent (weedier) than non-GMHT plants. Also greenhouse studies, in which the fitness of oilseed rape volunteers with no, single, or multiple herbicide tolerance was assessed, have shown no or little difference in fitness among oilseed rape plants in the absence of herbicide pressure (Simard et al., 2005). There is also no evidence that tolerance to glyphosate or glufosinate-ammonium enhances seed dormancy, and thus the persistence of GMHT oilseed rape plants, compared with their non-GM comparators (Hails et al., 1997; Sweet et al., 2004; Lutman et al., 2005, 2008; Messéan et al., 2007). Seed dormancy (secondary dormancy, as there is little primary dormancy at seed shed) is more likely to be affected by the genetic background of parental genotypes than the acquisition of herbicide tolerance traits (López-Granados and Lutman, 1998; Lutman et al., 2003; Gulden et al., 2004a,b; Gruber et al., 2004; Messéan et al., 2007; Baker and Preston, 2008). The evidence described above indicates that GMHT oilseed rape is neither more likely to survive nor to be more persistent or invasive than its non-GM comparator ~~in~~ the absence of glyphosate- or glufosinate-ammonium-containing herbicides.

A trait that is expected to exert a negative effect on the fitness of feral GMHT oilseed rape is male sterility (i.e. the absence of pollen-producing anthers), which occurs in a proportion of seed produced

by oilseed rape Ms8 × Rf3. Progeny may be male fertile or male sterile and have a variable number of copies of the *bar* gene, while a small proportion will have no *bar*, *barstar* or *barnase* genes. Male-sterile plants still produce stigmas and will set seed by pollen from another plant. They can therefore receive genes, but not transmit them via the pollen. The effect of such male sterility is to give high seed yields in selected oilseed rape varieties in fields, but it is not likely to increase the fitness of feral individuals and populations outside the field (Hails et al., 1997; Sweet et al., 2004; Lutman et al., 2005, 2008; Messéan et al., 2007).

Oilseed rape has hybridising wild relatives (section 6.1.1.2), but there is no evidence to suggest that herbicide tolerance traits in wild relatives change their behaviour (Norris et al., 2004; Warwick et al., 2008), or the scale and nature of their interactions with associated flora and fauna (Wilkinson and Ford, 2007). Progeny from hybrids of oilseed rape and wild relatives bearing the herbicide tolerance trait does not show any enhanced fitness, persistence or invasiveness and behaves as its non-GM comparators, unless the herbicides for which tolerance has been obtained are applied (Londo et al., 2010; Watrud et al., 2011).

The EFSA GMO Panel has reviewed all relevant scientific literature that has been published since the adoption of its scientific opinion in 2005 and concludes that no new information has become available that would require reconsideration of its previous conclusion on oilseed rape Ms8, Rf3 and Ms8 × Rf3 (see EFSA, 2005a, 2009c). Therefore, the conclusion that oilseed rape Ms8 × Rf3 has no altered agronomic and phenotypic characteristics, except for the herbicide tolerance, is reiterated. Glufosinate-ammonium-tolerant oilseed rape is neither more likely to survive, nor be more persistent or invasive, than its non-GM comparators in the absence of glufosinate-ammonium-containing herbicides. The ability of oilseed rape to successfully invade and subsequently persist in ruderal habitats appears to be limited principally by the availability of seed germination sites and interspecific plant competition, and there is no evidence that genes conferring herbicide tolerance significantly alter its competitive ability, except in the presence of the herbicidal active ingredient. The likelihood of unintended environmental effects due to the establishment, survival and spread of oilseed rape Ms8, Rf3 and Ms8 × Rf3 will therefore not be different from that of commercial oilseed rape varieties, unless exposed to glufosinate- ammonium-containing herbicides.

#### 6.1.1.2. Potential for gene transfer[31]

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 the dispersal of pollen and seed.

(a) Plant-to-bacteria gene transfer

The EFSA GMO Panel previously evaluated the plant-to-bacteria gene transfer from oilseed rape Ms8, Rf3 and Ms8 × Rf3 to bacteria and the potential environmental consequences of such gene transfer (EFSA, 2005a, 2009a). The EFSA GMO Panel concluded that "in the very unlikely event that such a horizontal gene transfer would take place, no adverse effects on human and animal health or the environment are expected, as no principally new traits would be introduced into or expressed by natural microbial communities" (EFSA, 2009a).

The EFSA GMO Panel reiterates its previous conclusions, as it did not identify properties of the inserted DNA in oilseed rape Ms8, Rf3 and Ms8 × Rf3 that would change its likelihood of horizontal transfer compared with other plant genes. Current scientific knowledge (see EFSA, 2009b for further details) suggests that gene transfer from GM plants to bacteria under natural conditions is extremely unlikely and that its establishment into the recipient genomes would occur primarily through homologous recombination. The *bar, barnase* and *barstar* genes, as expressed in oilseed rape Ms8 × Rf3, are of bacterial origin (from *Bacillus amyloliquefaciens* and *Streptomyces hygroscopicus*,

[31] Technical dossier / Sections D6 and D9.3.

respectively). As natural variants of such genes are already present in bacteria in the environment, homologous recombination and acquisition of the recombinant genes by bacteria will not confer novel properties possibly providing selective advantages to members of the natural microbial communities. In environments frequently exposed to glufosinate-ammonium, bacteria with resistance to this compound may be selected. However, glufosinate-ammonium tolerance and resistance has been described for several bacterial species and is expected to be common in bacterial communities in the environment (Bartsch and Tebbe, 1989; Mohr and Tebbe, 2007). Considering the scope of this application, it should be noted that glufosinate-ammonium as a herbicidal compound and selective agent for some bacteria is not expected to be present in the main receiving environment, i.e. the gastrointestinal tract of humans. Taking into account the bacterial origin of the *barnase*, *barstar* and *bar* genes including the activities of their encoded proteins, the limited exposure indicated by the scope of this application, and a highly unlikely but theoretically possible horizontal transfer of these recombinant genes in the background of natural variants of these genes and natural gene transfer processes between bacteria in the environment, potentially adverse effects on human health or the environment are not expected.

Considering the intended uses as food and the above assessment, and in agreement with its previous scientific opinions on oilseed rape Ms8, Rf3 and Ms8 × Rf3, the EFSA GMO Panel has not identified any concern associated with horizontal gene transfer from oilseed rape Ms8, Rf3 and Ms8 × Rf3 to bacteria.

(b) Plant-to-plant gene transfer

The EFSA GMO Panel has previously evaluated the plant-to-plant gene transfer from feral oilseed rape Ms8, Rf3 and Ms8 × Rf3 plants to cross-compatible plant species and the potential environmental consequences of such gene transfer (EFSA, 2005a, 2009c). The EFSA GMO Panel indicated that "spilled seeds could result in escaped GM plants that survive and establish which could outcross and disperse genes to other plants or plant species. However, if gene flow or escape into the environment occurs the events would only show enhanced fitness in the presence of the complementary herbicide as demonstrated for herbicide tolerant GT73 oilseed rape" (EFSA, 2005a).

Newly published data since the adoption of the 2005 EFSA GMO Panel's scientific opinion confirm that seed dispersal is likely to occur resulting in feral GMHT oilseed rape plants in regions where GMHT oilseed rape is cultivated and/or transported (reviewed by Devos et al., 2012). In regions where GMHT oilseed rape is widely grown such as western Canada and the USA, monitoring surveys revealed the widespread occurrence of feral GMHT oilseed rape plants along field margins of agricultural fields, as well as along transport routes (such as road verges and railway lines). In the study by Yoshimura et al. (2006), approximately two-thirds of the feral plants sampled were transgenic, whereas all feral plants sampled by Knispel et al. (2008) exhibited the glyphosate or glufosinate-ammonium tolerance traits (or both). In North Dakota (USA), 80 % (231/288) of the sampled feral oilseed rape plants expressed at least one herbicide trait (CP4 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) and phosphinothricin-N-acetyltransferase (PAT)): 41 % (117/288) of the plants were positive only for CP4 EPSPS and 39 % (112/288) were positive for PAT; and 0.7 % (2/288) expressed both herbicide tolerance traits (Schafer et al., 2011). The presence of feral GMHT oilseed rape plants was also detected at the port of Vancouver on the west coast of Canada, where most GMHT oilseed rape seed for export is transported by rail (Yoshimura et al., 2006). These data indicate that feral GMHT oilseed rape will be present along roadsides and other ruderal habitats in areas where GMHT oilseed rape is commercially grown and transported as viable seed. Surveys in Japan, where GMHT oilseed rape is currently not grown commercially, performed in and around major ports and along roads leading from these ports to inland processing facilities, reported feral oilseed rape plants with glyphosate or glufosinate-ammonium tolerance, and to a lesser extent both traits (Saji et al., 2005; Aono et al., 2006; Kawata et al., 2009; Nishizawa et al., 2009). The proportion of feral plants that was transgenic varied substantially across years and sampling sites, ranging from 0.2 % to 100 % (Kawata et al., 2009; Nishizawa et al., 2009). Aono et al. (2006) also reported the presence of *barnase* and *barstar* genes in the progeny of some of the sampled oilseed rape plants. As

no GM oilseed rape has been grown for marketing purposes in Japan (Nishizawa et al., 2010), transgene presence could be attributed to the accidental loss and spillage of imported viable GMHT oilseed rape seeds. These data indicate that seed dispersal of GMHT oilseed rape will occur wherever it is transported or cultivated, so that feral plants are likely to be present along transport routes in all countries cultivating and/or receiving imports of viable seeds of GMHT oilseed rape and in ruderal habitats in areas where GMHT oilseed rape is commercially grown.

Oilseed rape is an outcrossing species with the potential to cross-pollinate other oilseed rape types at varying levels of frequency depending on flowering synchrony, spatial arrangement of plants, presence of pollinator insects and other factors as reviewed by Eastham and Sweet (2004). Feral oilseed rape Ms8 × Rf3 plants arising from spilled seeds could therefore pollinate crop plants of non-GM oilseed rape if feral populations are immediately adjacent to field crops (Garnier and Lecomte, 2006). Shed seed from cross-pollinated crop plants could emerge as GM volunteers in subsequent crops. Squire et al. (2011) and Devos et al. (2012) considered that the frequency of such events was likely to be extremely low and concluded that this route of gene flow would not introduce significant numbers of GM plants into farmland or result in any environmental consequences.

Oilseed rape is known to spontaneously hybridise with certain of its sexually compatible wild relatives (Scheffler and Dale, 1994; Eastham and Sweet, 2004; Chèvre et al., 2004; Devos et al., 2009). Several oilseed rape × wild relative hybrids have been reported in the scientific literature, but under field conditions transgene introgression has been confirmed only for progeny of oilseed rape × *Brassica rapa* hybrids (Hansen et al., 2001, 2003; Warwick et al., 2003, 2008; Norris et al., 2004; Jørgensen, 2007). Due to ecological and genetic barriers, not all relatives of oilseed rape share the same potential for hybridisation and transgene introgression (Jenczewski et al., 2003; Chèvre et al., 2004; FitzJohn et al., 2007; Wilkinson and Ford, 2007; Devos et al., 2009; Jørgensen et al., 2009). As no or only very low numbers of viable and fertile hybrids are obtained between oilseed rape and most of its wild relatives under ideal experimental conditions (e.g. through the use of artificial pollination and embryo rescue techniques in laboratory conditions (see FitzJohn et al., 2007)), Wilkinson et al. (2003) concluded that exposure under real conditions is likely to be negligible, and the probability of transgene introgression is extremely small in most instances, with the exception of *B. rapa* in areas where it occurs close to oilseed rape. Transgene introgression is likely to take place when oilseed rape and *B. rapa* grow in close proximity over successive growing seasons, especially if no significant fitness costs are imposed to backcross plants by transgene acquisition (Snow et al., 1999). However, hybrids between *B. napus* and *B. rapa* are mostly triploid with low male fertility, and hence low ability to pollinate and form backcrosses with *B. napus* (Norris et al., 2004). Incidences of hybrids and backcrosses with *B. rapa* were found to be low in fields in Denmark (Jørgensen et al., 2004) and the UK (Norris et al., 2004). Recent observations in Canada confirmed the persistence of a glyphosate tolerance trait over a period of 6 years in a population of *B. rapa* in the absence of herbicide pressure (with the exception of possible exposure to glyphosate in one year) and in spite of fitness costs associated with hybridisation (Warwick et al., 2008). A single GM *B. rapa* ×*B. napus* hybrid was also reported along a road in Vancouver (Yoshimura et al., 2006), confirming the hybridisation possibility between these two *Brassica* species, albeit at very low frequencies. However, Elling et al. (2009) measured the extent of hybridisation between autotetraploid *B. rapa* varieties (female) and *B. napus* (pollen donor) under experimental field conditions and found that hybridisation with tetraploid *B. rapa* seemed to be more likely than with diploid *B. rapa*. They reported that male fertility was higher in these hybrids than those formed with diploid *B. rapa* and suggested that introgression frequencies from *B. napus* to *B. rapa* would be higher in tetraploid *B. rapa*. They also reported the presence of some feral tetraploid *B. rapa* populations in north-west Germany, but did not report on interspecific hybrids or backcrosses in these populations.

Surveys and analyses conducted in Japan did not detect transgenes in seed collected from wild relatives (*B. rapa* and *B. juncea*) sampled at several ports and along roadsides and riverbanks (Saji et al., 2005; Aono et al., 2006). Introgression of genetic material from feral oilseed rape to wild relatives, while theoretically possible, is likely to be very low due to the combined low conditional probabilities

of spillage of GMHT oilseed rape in areas where wild relatives (e.g. *B. rapa*) are present, of germination given spillage, of survival of oilseed rape plants given germination, of hybridisation with its wild relatives given survival, and of the survival and the low fertility of interspecific hybrids themselves, all of which restrict backcrossing with the wild relative.

Glufosinate-ammonium-containing herbicides are used for general weed control in orchards and around field margins, banks and ditches, and could encourage increased persistence of glufosinate-ammonium-tolerant plants in these areas. In such areas, the glufosinate ammonium tolerance trait is likely to increase the fitness of GMHT plants (be it feral plants or progeny from hybrids of oilseed rape and wild relatives) relative to plants not tolerant to glufosinate ammonium when exposed to glufosinate-ammonium-containing herbicides (Londo et al., 2010, 2011; Watrud et al., 2011). However, both the occurrence of feral GMHT oilseed rape resulting from seed import spills and the introgression of genetic material from feral oilseed rape to wild relatives are likely to be low in an import scenario. Therefore, feral oilseed rape plants and genes introgressed into other cross-compatible plants would not create significant agronomic or environmental impacts, even after exposure to glufosinate-ammonium-containing herbicides.

Having reviewed all relevant scientific literature published since the adoption of its scientific opinion in 2005, the EFSA GMO Panel confirms that feral GMHT oilseed rape plants are likely to occur wherever GMHT oilseed rape is transported. However, as indicated in section 6.1.1.1, there is no evidence that the herbicide tolerance trait results in enhanced fitness, persistence or invasiveness of oilseed rape Ms8, Rf3 and Ms8 × Rf3, or hybridising wild relatives, unless they are exposed to glufosinate-ammonium-containing herbicides. Feral oilseed rape plants and genes introgressed into other cross-compatible plants would not create significant agronomic or environmental impacts, even after exposure to glufosinate-ammonium-containing herbicides.

#### 6.1.1.3. Potential interactions of the GM plant with target organisms[32]

Interactions of oilseed rape Ms8, Rf3 and Ms8 × Rf3 with target organisms are not considered an issue by the EFSA GMO Panel, as there are no target organisms.

#### 6.1.1.4. Potential interactions of the GM plant with non-target organisms[33]

Owing to the intended uses of oilseed rape Ms8, Rf3 and Ms8 × Rf3, which exclude cultivation, and the low levels of exposure to the environment, potential interactions of the GM plant with non-target organisms are not considered an issue by the EFSA GMO Panel. Furthermore, there are no indications that the expression of the PAT protein in glufosinate-ammonium-tolerant crops will cause direct adverse effects on non-target organisms (CERA, 2011).

#### 6.1.1.5. Potential interactions with the abiotic environment and biogeochemical cycles[34]

Owing to the intended uses of oilseed rape Ms8, Rf3 and Ms8 × Rf3, which exclude cultivation, and the low levels of exposure to the environment, potential interactions with the abiotic environment and biogeochemical cycles are not considered an issue by the EFSA GMO Panel.

### *6.1.2. Post-market environmental monitoring*[35]

The objectives of a monitoring plan, according to Annex VII of Directive 2001/18/EC, are: (i) to 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 (ii) to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment that were not anticipated in the environmental risk assessment. Monitoring is related to risk management, and the final adoption

[32] Technical dossier / Section D9.4.
[33] Technical dossier /Section D9.5.
[34] Technical dossier / Sections D9.8 and D10.
[35] Technical dossier / Section D11.

of the monitoring plan falls outside the mandate of EFSA. However, the EFSA GMO Panel gives its opinion on the scientific content of the monitoring plan provided by the applicant (EFSA, 2006b, 2011a).

The scope of the monitoring plan provided by the applicant is in line with the intended uses of oilseed rape Ms8, Rf3 and Ms8 × Rf3. As the scope of the application EFSA-GMO-BE-2010-81 does not include cultivation, the environmental risk assessment was concerned with the accidental release into the environment of viable seeds of oilseed rape Ms8, Rf3 and Ms8 × Rf3 unintentionally present in food, and with the horizontal gene transfer to bacteria occurring in the environment or human digestive tract. The environmental risk assessment identified no potential adverse effects to the environment. Therefore, no case-specific monitoring is necessary.

The general surveillance plan proposed by the applicant includes: (i) the description of an approach involving operators (federations involved in oilseed rape import and processing) reporting to the applicant via a centralised system any observed adverse effect(s) of GMOs on human health and the environment; (ii) a coordinating system established by EuropaBio for the collection of the information recorded by the various operators; and (iii) the use of networks of existing surveillance systems (Lecoq et al., 2007; Windels et al., 2008). The applicant proposes to submit a general surveillance report on an annual basis and a final report at the end of the consent period.

The EFSA GMO Panel considers that the scope of the monitoring plan proposed by the applicant is in line with the intended uses of oilseed rape Ms8, Rf3 and Ms8 × Rf3, as the environmental risk assessment does not cover cultivation and identified no potential adverse environmental effects. In addition, the EFSA GMO Panel acknowledges the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure in the case of accidental release of viable seeds of oilseed rape Ms8, Rf3 and Ms8 × Rf3. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

### 6.2. Conclusion

Considering the intended uses of oilseed rape Ms8, Rf3 and Ms8 × Rf3, which exclude cultivation, the environmental risk assessment is concerned with the accidental release into the environment of viable seeds of oilseed rape Ms8, Rf3 and Ms8 × Rf3 unintentionally present in food, and with horizontal gene transfer to bacteria occurring in the environment or human digestive tract. In the case of accidental release into the environment of viable oilseed rape Ms8, Rf3 and Ms8 × Rf3 seeds, there are no indications of an increased likelihood of establishment and spread of feral oilseed rape Ms8, Rf3 and Ms8 × Rf3 plants, or hybridising relatives, unless exposed to glufosinate-ammonium-containing herbicides. The low levels of environmental exposure of oilseed rape Ms8, Rf3 and Ms8 × Rf3 plants indicate that the risk to non-target organisms is extremely low. Due to its intended uses, only a low level of exposure of bacteria in the environment, including those in the gastrointestinal tract, to recombinant DNA from oilseed rape Ms8, Rf3 and Ms8 × Rf3 is expected. The unlikely but theoretically possible transfer of the recombinant genes from oilseed rape Ms8, Rf3 and Ms8 × Rf3 to bacteria does not raise concerns owing to the lack of any selective advantage that would be given to the recipients in the receiving environments. The scope of the PMEM provided by the applicant and the reporting intervals are in line with the intended uses of oilseed rape Ms8, Rf3 and Ms8 × Rf3 and the EFSA GMO Panel scientific opinions providing guidance on the PMEM of GM plants (EFSA, 2006b, 2011a). In addition, the EFSA GMO Panel acknowledges the approach proposed by the applicant of putting in place appropriate management systems to restrict environmental exposure in cases of accidental release of viable seeds of oilseed rape Ms8, Rf3 and Ms8 × Rf3. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

## OVERALL CONCLUSIONS AND RECOMMENDATIONS

The EFSA GMO Panel was requested to carry out a scientific risk assessment of oilseed rape Ms8, Rf3 and Ms8 × Rf3 for food containing or consisting of, and food produced from or containing ingredients produced from, oilseed rape Ms8, Rf3 and Ms8 × Rf3 (with the exception of processed oil) in accordance with Regulation (EC) No 1829/2003. In evaluating oilseed rape Ms8, Rf3 and Ms8 × Rf3, the EFSA GMO Panel considered the information in the application EFSA-GMO-BE-2010-81, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications. Further information from previous applications for placing on the market under EU regulatory procedures the oilseed rape Ms8, Rf3 and Ms8 × Rf3 was taken into account.

Molecular analysis confirmed that the Ms8 and Rf3 inserts are present and that their integrity is retained in oilseed rape Ms8 × Rf3. Results of the bioinformatic analyses of the flanking sequences and the open reading frames spanning the insert–genomic DNA junctions did not reveal safety issues. The levels of the PAT protein in oilseed rape Ms8, Rf3 and Ms8 × Rf3 have been sufficiently analysed. The EFSA GMO Panel considers that the molecular characterisation of oilseed rape Ms8, Rf3 and Ms8 × Rf3 does not indicate a safety concern.

The comparative analysis indicated that no biologically relevant differences were identified in the compositional, agronomic or phenotypic characteristics of oilseed rape Ms8 × Rf3 compared with its non-GM comparator oilseed rape, except for the newly expressed PAT, barnase and barstar proteins.

The safety of the newly expressed proteins present in oilseed rape Ms8, Rf3 and Ms8 × Rf3 were previously assessed by the EFSA GMO Panel in 2005 and 2009, and no safety concerns were identified for humans and animals, in the context of their intended uses. A 42-day broiler feeding study confirmed that oilseed rape Ms8 × Rf3 was as nutritious as its non-GM comparator. The new information provided in the present application does not raise concerns regarding the toxicity and allergenicity of oilseed rape Ms8, Rf3 and Ms8 × Rf3. Thus, the EFSA GMO Panel reiterates its previous conclusions that oilseed rape Ms8, Rf3 and Ms8 × Rf3 are unlikely to have an adverse effect on human and animal health, in the context of their intended uses.

As this application does not cover cultivation of oilseed rape Ms8, Rf3 and Ms8 × Rf3, there is no requirement for scientific information on possible environmental effects associated with the cultivation of oilseed rape Ms8, Rf3 and Ms8 × Rf3. In the event of the accidental release into the environment of viable oilseed rape Ms8, Rf3 and Ms8 × Rf3 seeds unintentionally present in food, there are no indications of an increased likelihood of establishment and spread of feral oilseed rape Ms8, Rf3 and Ms8 × Rf3, unless exposed to glufosinate-ammonium-containing herbicides. Likewise, evidence indicates that hybridising wild relatives that may theoretically have acquired the herbicide tolerance trait through vertical gene flow are neither more likely to establish nor to spread than their non-GM comparators in the absence of glufosinate-ammonium-containing herbicides. Considering the intended uses of oilseed rape Ms8, Rf3 and Ms8 × Rf3, potential interactions of feral oilseed rape Ms8, Rf3 and Ms8 × Rf3 plants with the biotic and abiotic environment are not considered to be an issue owing to the low levels of exposure. Due to the intended uses of oilseed rape Ms8, Rf3 and Ms8 × Rf3, only a low level exposure of bacteria in the environment, including those in the gastrointestinal tract, to recombinant DNA from oilseed rape Ms8, Rf3 and Ms8 × Rf3 is expected. The unlikely but theoretically possible transfer of the recombinant genes from oilseed rape Ms8, Rf3 and Ms8 × Rf3 to bacteria does not raise concerns owing to the lack of any selective advantage that would be given to the recipients in the receiving environments. In addition, tolerance and resistance to glufosinate-ammonium is widespread among bacteria in the environment, making it unlikely that horizontal gene transfer would add to this natural background. The scope of the PMEM plan provided by the applicant is in line with the intended uses of oilseed rape Ms8, Rf3 and Ms8 × Rf3. Furthermore, the EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in its general surveillance plan.

In conclusion, the EFSA GMO Panel considers that the information available for oilseed rape Ms8, Rf3 and Ms8 × Rf3 addresses the scientific issues described in its relevant guidance documents and the scientific comments raised by the Member States, and that the oilseed rape Ms8, Rf3 and Ms8 × Rf3 are unlikely to have an adverse effect on human and animal health or on the environment, in the context of their intended uses.

## DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of Belgium, received on 23 June 2010, concerning a request for placing on the market of oilseed rape Ms8, Rf3 and Ms8 × Rf3 in the European Union in accordance with Regulation (EC) No 1829/2003.

2. Acknowledgement letter, dated 7 July 2010, from EFSA to the Competent Authority of Belgium (Ref. CGL/RM/PB/KL/mt (2010) 4959896).

3. Letter from EFSA to the applicant, dated 3 August 2010, requesting additional information under completeness check (Ref. KL/CE/AFD/shv (2010) 5043788).

4. Letter from the applicant, received on 16 September 2011, providing additional information under completeness check.

5. Letter from EFSA to the applicant, dated 5 October 2011, delivering the "Statement of Validity" for application EFSA-GMO-BE-2010-81, oilseed rape Ms8, Rf3 and Ms8 × Rf3 submitted by Bayer BioScience under Regulation (EC) No 1829/2003 (Ref. EW/ZD/CE/lg (2011) 6008941).

6. Letter from the applicant, received on 19 October 2011, providing EFSA with an updated version of the application EFSA-GMO-BE-2010-81 submitted by Bayer BioScience under Regulation (EC) No 1829/2003.

7. Letter from EFSA to the applicant, dated 29 November 2011, requesting additional information and stopping the clock (Ref. EW/ZD/AFD/shv (2011) 6099213).

8. Letter from EFSA to the applicant, dated 25 January 2012, requesting additional information (Ref. EW/ZD/AFD/mt (2012) 6199074).

9. Letter from the applicant to EFSA, received on 3 April 2012, providing additional information.

10. Letter from EFSA to the applicant, dated 24 May 2012, requesting additional information (Ref. EW/ZD/AFD/cz (2012) 6604887).

11. Letter from the applicant to EFSA, received on 29 May 2012, providing additional information.

12. Letter from EFSA to the applicant, dated 11 July 2012, restarting the clock (Ref. EW/ZD/AFD/shv (2012) 6717253).

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