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Scientific Opinion on application EFSA-GMO-NL-2013-119 for authorisation of genetically modified glufosinate-ammonium- and glyphosate-tolerant oilseed rape MON 88302 × MS8 × RF3 and subcombinations independently of their origin, for food and feed uses, import and processing submitted in accordance with Regulation (EC) No 1829/2003 by Monsanto Company and Bayer CropScience

EFSA Panel on Genetically Modified Organisms (GMO),
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Abstract

In this opinion, the GMO Panel assessed the three-event stack oilseed rape (OSR) MON 88302 × MS8 × RF3 and its three subcombinations, independently of their origin. The GMO Panel has previously assessed the single events combined to produce this three-event stack OSR and did not identify safety concerns; no new information that would modify the original conclusions was identified. The combination of the single OSR events and of the newly expressed proteins in the three-event stack OSR does not give rise to food and feed safety and nutrition issues – based on the molecular, agronomic/phenotypic and compositional characteristics. In the case of accidental release of viable OSR MON 88302 × MS8 × RF3 seeds into the environment, the three-event stack OSR would not raise environmental safety concerns. The GMO Panel therefore concluded that the three-event stack OSR is as safe and as nutritious as its conventional counterpart and the tested non-GM reference varieties in the context of the scope of this application. Since no new safety concerns were identified for the previously assessed two-event stack OSR MS8 × RF3, the GMO Panel considered that its previous conclusions on this subcombination remain valid. For the two subcombinations MON 88302 × MS8 and MON 88302 × RF3 for which no experimental data were provided, the GMO Panel assessed the likelihood of interactions among the single events, and concluded that their different combinations would not raise safety concerns. These two subcombinations are therefore expected to be as safe as the single events, the previously assessed OSR MS8 × RF3, and OSR MON 88302 × MS8 × RF3. Since the post-market environmental monitoring plan for the three-event stack OSR does not include any provisions for two subcombinations not previously assessed, the GMO Panel recommended the applicant to revise the plan accordingly.

Keywords: GMO, oilseed rape (*Brassica napus*), MON 88302 × MS8 × RF3, herbicide tolerance, Regulation (EC) No 1829/2003

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Summary

Following the submission of application EFSA-GMO-NL-2013-119 under Regulation (EC) No 1829/2003[1] by Monsanto Company and Bayer CropScience (referred to hereafter as the applicant), the Panel on Genetically Modified Organisms of the European Food Safety Authority (referred to hereafter as GMO Panel) was asked to deliver a scientific opinion on the safety of genetically modified glufosinate-ammonium- and glyphosate-tolerant oilseed rape MON 88302 × MS8 × RF3 (referred to hereafter as 'three-event stack oilseed rape') and its subcombinations[2] (referred to hereafter as 'subcombinations independently of their origin' according to the Commission Implementing Regulation (EU) No 503/2013[3]). The scope of application EFSA-GMO-NL-2013-119 is for the placing on the market of oilseed rape MON 88302 × MS8 × RF3 and subcombinations MON 88302 × MS8, MON 88302 × RF3 and MS8× RF3, independently of their origin, for food and feed uses, import and processing.

The term 'subcombination' refers to any combination of two of the events present in the three-event stack oilseed rape. The safety of subcombinations occurring as segregating progeny in the harvested seeds of oilseed rape MON 88302 × MS8 × RF3 is evaluated in the context of the assessment of the three-event stack oilseed rape in Section 3.3 of the present GMO Panel Scientific Opinion. The safety of subcombinations that have either been, or could be produced by conventional crossing through targeted breeding approaches, and which can be bred, produced and marketed independently of the three-event stack, are risk assessed in the Section 3.4 of the present GMO Panel Scientific Opinion.

In delivering its Scientific Opinion, the GMO Panel considered the data available on the single events, the three-event stack oilseed rape and the previously risk assessed subcombination MS8 × RF3, the scientific comments submitted by the Member States and the relevant scientific literature. The three-event stack oilseed rape was produced by conventional crossing to combine three single oilseed rape events MON 88302, expressing the 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein for tolerance to glyphosate-containing herbicide; MS8, expressing Barnase and phosphinothricin acetyltransferase (PAT) proteins, and RF3, expressing Barstar and PAT proteins, for tolerance to glufosinate-ammonium-containing herbicides and for obtaining heterosis (hybrid vigour).

The GMO Panel evaluated the three-event stack oilseed rape and its subcombinations with reference to the scope of this application and appropriate principles described in its guidelines for the risk assessment of genetically modified (GM) plants and derived food and feed, the environmental risk assessment of GM plants and the post-market environmental monitoring (PMEM) of GM plants. The GMO Panel guidance documents applicable to this application establish the principle that where all single events have been assessed, the risk assessment of stacked events should focus mainly on issues related to: (a) stability of the events; (b) expression of the events; and (c) potential interactions resulting from the combination of the events.

For application EFSA-GMO-NL-2013-119, previous assessments of the three single oilseed rape events (MON 88302, MS8 and RF3) and of the two-event stack oilseed rape MS8 × RF3 provided a basis to evaluate the three-event stack oilseed rape and its subcombinations. Oilseed rape MON 88302, MS8, RF3 and MS8 × RF3 were previously assessed by the GMO Panel and no concerns on their safety were identified. No safety issue concerning the three single oilseed rape events was identified by the updated bioinformatic analyses, nor reported by the applicant since the publication of the previous GMO Panel Scientific Opinions. Therefore, the GMO Panel considers that its previous conclusions on the safety of the single events remain valid.

For the three-event stack oilseed rape, the risk assessment included the molecular characterisation of the inserted DNA and analysis of protein expression. An evaluation of the comparative analyses of agronomic/phenotypic and compositional characteristics was undertaken, and the safety of the newly expressed proteins and the whole food/feed were evaluated with respect to potential toxicity, allergenicity and nutritional characteristics. An evaluation of environmental impacts and PMEM plans was also undertaken.

[1] 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, L 268, p. 1–23.

[2] The subcombinations are two-event stacks MON 88302 × MS8, MON 88302 × RF3 and MS8 × RF3 oilseed rape.

[3] Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. OJ L157, 8.6.2013, p. 1–48.

The molecular data establish that the events stacked in oilseed rape MON 88302 × MS8 × RF3 have retained their integrity. Protein expression analyses showed some difference between the levels in the single lines and those in the three-event stack, which were not unexpected. No indications of interactions that may affect the integrity of the events and the levels of the newly expressed proteins in this triple-event stack oilseed rape were identified.

No relevant differences between oilseed rape MON 88302 × MS8 × RF3 and its conventional counterpart requiring further assessment regarding food and feed safety and environmental impact were identified in seed composition and agronomic and phenotypic characteristics tested.

Based on the molecular, agronomic, phenotypic or compositional characteristics, the combination of oilseed rape events MON 88302, MS8 and RF3 in the three-event stack oilseed rape did not give rise to issues – regarding food and feed safety and nutrition. The combination of the newly expressed proteins in the three-event stack oilseed rape did not raise concerns for human and animal health.

In the case of accidental release into the environment of viable seeds of the three-event stack oilseed rape, there are no indications of an increased likelihood of establishment and spread of feral oilseed rape MON 88302 × MS8 × RF3 plants, or hybridising wild relatives, unless these plants are exposed to glufosinate-ammonium- and/or glyphosate-containing herbicides. However, the GMO Panel is of the opinion that the latter will not result in different environmental impacts compared to conventional oilseed rape. Considering the scope of application EFSA-GMO-NL-2013-119, interactions with the biotic and abiotic environment were not considered to be a relevant issue. Risks associated with an unlikely but theoretically possible horizontal gene transfer of recombinant DNA from the three-event stack oilseed rape to bacteria have not been identified. Considering the introduced traits, the outcome of the comparative analysis, the routes of exposure and limited exposure levels, the GMO Panel concludes that the three-event stack oilseed rape would not raise environmental safety concerns in the case of accidental release of viable GM oilseed rape seeds into the environment, irrespective of possible interactions between the individual events within this three-event stack oilseed rape.

The GMO Panel concludes that oilseed rape MON 88302 × MS8 × RF3 is as safe and as nutritious as its conventional counterpart and the tested non-GM oilseed rape reference varieties in the context of the scope of this application.

Since no safety concerns were identified for the previously assessed two-event stack oilseed rape MS8 × RF3, and no new data leading to modification of the original conclusions on safety were identified, the GMO Panel considers that its previous conclusions on this subcombination remain valid. For the two subcombinations MON 88302 × MS8 and MON 88302 × RF3, for which no experimental data were provided, the GMO Panel assessed possible interactions between the events, and concludes that different combinations of the events MON 88302, MS8 and RF3 in oilseed rape would not raise safety concerns. These two subcombinations are therefore expected to be as safe as the single events, the previously assessed two-event oilseed rape stack MS8 × RF3, and the three-event stack oilseed rape MON 88302 × MS8 × RF3.

Given the absence of safety concerns identified on food and feed derived from oilseed rape MON 88302 × MS8 × RF3 and its subcombinations MS8 × RF3, MON 88302 × MS8 and MON 88302 × RF3, the GMO Panel considers that post-market monitoring of these products is not necessary.

The GMO Panel considers that the scope of the PMEM plans provided by the applicant is consistent with the scope of the three-event stack oilseed rape and the already assessed two-event stack oilseed rape MS8 × RF3. The GMO Panel agrees with the reporting intervals proposed by the applicant in the PMEM plans. However, the PMEM plan submitted by the applicant for the three-event stack oilseed rape does not include any provisions for two subcombinations that were not previously assessed. Therefore, the GMO Panel recommends the applicant to revise the plan accordingly.

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1. Introduction

1.1. Background

On 5 December 2013, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands application EFSA-GMO-NL-2013-119, for authorisation of genetically modified (GM) glufosinate-ammonium- and glyphosate-tolerant oilseed rape MON 88302 × MS8 × RF3 (referred to hereafter as three-event stack oilseed rape), submitted by Monsanto Company and Bayer CropScience (referred to hereafter as the applicant) within the framework of Regulation (EC) No 1829/2003, for food and feed uses, import and processing. The risk assessment of application EFSA-GMO-NL-2013-119 presented here is for the placing on the market of three-event stack oilseed rape and subcombinations (MS8 × RF3, MON 88302 × MS8 and MON 88302 × RF3), independently of their origin, for food and feed uses, import and processing.

After receiving application EFSA-GMO-NL-2013-119 and in accordance with Articles 5(2)(b) and 17 (2)(b) of Regulation (EC) No 1829/2003, EFSA informed Member States and the European Commission, and made the summary of the application available to the public on the EFSA website.[4] 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. EFSA requested additional information under completeness check on 28 January 2014 and received it on 31 March 2014. On 24 April 2014, EFSA declared the application valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003. The clock of the application was stopped from 24 April 2014 to 22 May 2014 due to the pending assessment of the single-event oilseed rape MON 88302 (application reference EFSA-GMO-BE-2011-101).

EFSA made the valid application available to Member States and the European Commission, and consulted nominated risk assessment bodies of Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC[5] following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Member States had 3 months after the date of receipt of the valid application (until 17 September 2014[6]) to make their opinion known.

The GMO Panel carried out the scientific risk assessment of the three-event stack oilseed rape and subcombinations MON 88302 × MS8, MON 88302 × RF3 and MS8 × RF3 (referred to as 'subcombinations independently of their origin' according to the Commission Implementing Regulation (EU) No 503/2013). The GMO Panel requested additional information from the applicant on 3 July 2014, 17 October 2014, 23 June 2015, 11 February 2016, 16 February 2016 and 8 July 2016. The applicant provided the requested information on 1 September 2014, 15 December 2014, 21 September 2015, 30 March 2016, 11 July 2016 and 27 September 2016, respectively. The applicant provided additional information spontaneously on 13 December 2016.

In the frame of contract OC/EFSA/UNIT/GMO/2013/01 and OC/EFSA/UNIT/GMO/2014/01, the contractors performed preparatory work and delivered reports on the methods applied by the applicant in performing bioinformatic analyses and statistical analyses respectively.

In giving its Scientific Opinion to the European Commission, 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 6 months from the acknowledgement of the valid application. As additional information was requested by the GMO Panel, the time limit of 6 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).

1.2. Terms of Reference as provided by the requestor

The GMO Panel was asked to carry out a scientific assessment of oilseed rape 'MON 88302 × MS8 × RF3 and all subcombinations of the individual events independently of their origin (as present in the segregating progeny as well as independent stacks to be placed on the

[4] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2013-01002

[5] 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. OJ L 106, 12.3.2001, p. 1–38.

[6] The 3-month commenting period on application EFSA-GMO-NL-2013-119 started following the adoption by the EFSA GMO Panel of application EFSA-GMO-BE-2011-101 (oilseed rape MON 88302).

market as such)', for food and feed uses, import and processing in accordance with Articles 6(6) and 18(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 GMOs or food/feed 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 GMO Panel was not requested to give an opinion on information required under Annex II to the Cartagena Protocol. Furthermore, the GMO Panel did 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.

2. Data and methodologies

2.1. Data

In delivering its scientific opinion, the GMO Panel took into account application EFSA-GMO-NL-2013-119, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications.

2.2. Methodologies

The GMO Panel carried out a scientific risk assessment of oilseed rape MON 88302 × MS8 × RF3 and its subcombinations MON 88302 × MS8, MON 88302 × RF3, MS8 × RF3, independently of their origin (Table 1), for food and feed uses, import and processing in accordance with Articles 6(6) and 18 (6) of Regulation (EC) No 1829/2003. The GMO Panel took into account the appropriate principles described in its guidelines for the risk assessment of GM plants and derived food and feed (EFSA GMO Panel, 2011a), the environmental risk assessment (ERA) of GM plants (EFSA GMO Panel, 2010a), and the post-market environmental monitoring (PMEM) of GM plants (EFSA GMO Panel, 2011b).

The comments raised by Member States are addressed in Annex G of EFSA's overall opinion and were taken into consideration during the scientific risk assessment.

3. Assessment

3.1. Introduction

Application EFSA-GMO-NL-2013-119 covers the three-event stack oilseed rape MON 88302 × MS8 × RF3 and its subcombinations MON 88302 × MS8, MON 88302 × RF3, MS8 × RF3 independently of their origin (Table 1). The scope of this application is for food and feed uses, import and processing, and excludes cultivation within the European Union (EU).

The term 'subcombination' refers to any combination of two of the events present in the three-event stack oilseed rape.

The safety of subcombinations occurring as segregating progeny in the harvested seeds of oilseed rape MON 88302 × MS8 × RF3 is evaluated in the context of the assessment of the three-event stack oilseed rape in Section 3.3 of the present GMO Panel Scientific Opinion.

'Subcombination' also covers combinations of two of the three events MON 88302, MS8 or RF3 that have either been, or could be produced by conventional crossing through targeted breeding approaches (EFSA GMO Panel, 2011a). These are oilseed rape stacks that can be bred, produced and marketed independently of the three-event stack oilseed rape. These stacks are risk assessed in the Section 3.4 of this GMO Panel Scientific Opinion.

The three-event stack oilseed rape was produced by conventional crossing to combine three single oilseed rape events MON 88302, expressing the 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein; MS8, expressing Barnase and phosphinothricin acetyltransferase (PAT) proteins; and RF3, expressing Barstar and PAT proteins.

Table 1: Oilseed rape stacks covered by the scope of application EFSA-GMO-NL-2013-119

Degree of stacking	Events	Unique identifiers
Three-event stack oilseed rape	MON 88302 × MS8 × RF3	MON-883Ø2-9 × ACSBNØØ5-8 × ACS-BNØØ3-6
Two-event stack oilseed rape	MON 88302 × MS8	MON-883Ø2-9 × ACSBNØØ5-8
	MON 88302 × RF3	MON-883Ø2-9 × ACS-BNØØ3-6
	MS8 × RF3	ACSBNØØ5-8 × ACS-BNØØ3-6

Herbicidal tolerance traits are achieved by the expression of CP4 EPSPS protein from *Agrobacterium* sp. strain CP4, and PAT from *Streptomyces hygroscopicus*. The expression of Barnase and Barstar proteins from *Bacillus amyloliquefaciens* constitutes the basis of a male fertility control system, through the use of the *barnase* gene, which removes male fertility in order to promote hybridisation, and the *barstar* gene which restores male fertility with oilseed rape lines MS8 and RF3 for obtaining heterosis (hybrid vigour).

The oilseed rape events MS8, RF3, MS8 × RF3 and MON 88302 have been previously assessed by the GMO Panel (Table 2), and no safety concerns were identified.

Table 2: Single- and two-event stacks oilseed rape previously assessed by the GMO Panel

Event	Application or mandate	EFSA Scientific Opinion
MON 88302	EFSA-GMO-BE-2011-101	EFSA GMO Panel (2014)
MS8, RF3 and MS8 × RF3	C/BE/96/01	EFSA (2005)
	EFSA-GMO-RX-MS8-RF3	EFSA (2009)
	EFSA-GMO-BE-2010-81	EFSA GMO Panel (2012)

EFSA guidance establishes the principle that 'For GM plants containing a combination of transformation events (stacked events) the primary concern for risk assessment is to establish that the combination of events is stable and that no interactions between the stacked events, that may raise safety concerns compared to the single events, occur. The risk assessment of GM plants containing stacked events focuses on issues related to: (a) stability of the inserts, (b) expression of the introduced genes and their products and (c) potential synergistic or antagonistic effects resulting from the combination of the events' (EFSA GMO Panel, 2011a).

3.2. Updated information on the events

Since the publication of the GMO Panel Scientific Opinions on the three single oilseed rape events (EFSA, 2005, 2009; EFSA GMO Panel, 2012, 2014), no safety issues pertaining to the single oilseed rape events have been reported by the applicant.

Updated bioinformatic analyses indicate that the RF3 insertion site interrupts a region that is covered by an expressed sequence tag (EST) in the vicinity of a region showing similarity to a rotundifolia-like 21 gene. The insert may have landed in the 3′ UTR of a rotundifolia-like 21 gene. The rotundifolia-like gene family encodes small peptides, some of which have been shown to regulate plant development (Valdivia et al., 2011). However, there is no indication from compositional or agronomic and phenotypic analyses that the possible deregulation of this gene has an effect on plant phenotype. Updated bioinformatic analyses on the junction regions for events MON 88302 and MS8 confirmed that no known endogenous genes were disrupted by any of the inserts.[7]

Updated bioinformatic analyses of the amino acid sequence of the newly expressed CP4 EPSPS and PAT proteins revealed no significant similarities to toxins and allergens.[7] In addition, updated bioinformatics analyses of the newly created open reading frames (ORFs) within the insert or spanning the junctions between the insert and genomic DNA, indicate that the expression of an ORF showing significant similarities to toxins and allergens is highly unlikely.[7]

In order to assess the possibility for horizontal gene transfer (HGT) by homologous recombination (HR), the applicant performed a sequence identity analysis for events MON 88302, MS8 and RF3. The assessment of these data and the potential consequences of plant-to-bacteria gene transfer are described in Section 3.3.4.2.

[7] Additional information 27 September 2016.

Based on the above information, the GMO Panel considers that its previous conclusions on the safety of the single oilseed rape events remain valid.

3.3. Risk assessment of the three-event stack oilseed rape MON 88302 × MS8 × RF3

3.3.1. Molecular characterisation

Possible interactions that would affect the integrity of the events, protein expression level or the biological functions conferred by the individual inserts are considered below.

3.3.1.1. Genetic elements and their biological function

The three-event stack oilseed rape was obtained by conventional crossing of events MON 88302, MS8 and RF3. The structure of the inserts introduced into oilseed rape events MON 88302, MS8 and RF3 is described in detail in the respective GMO Panel Scientific Opinions (Table 2), and no new genetic modifications were involved. Genetic elements in the expression cassettes of the single oilseed rape events are summarised in Table 3. Intended effects of the inserts in oilseed rape MON 88302 × MS8 × RF3 are summarised in Table 4.

Based on the known biological function of the newly expressed proteins (Table 4), the only foreseen interactions at the biological level are between the Barnase and Barstar proteins. The Barnase and Barstar proteins are expressed in plant tissues (i.e. tapetum cells of the flower buds only) that are not present in food or feed derived from the three-event stack oilseed rape.

Table 3: Genetic elements in the expression cassettes of the events stacked in the three-event stack oilseed rape MON 88302 × MS8 × RF3

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
MON 88302	P-*FMV/Tsf1* (*Figwort mosaic virus*)*	L-*Tsf1* (FMV)	TS-CTP2	CP4 *epsps* (*Agrobacterium* sp.)	*rbcS2 E9* (*Pisum sativum*)
MS8	*PssuAt* (*Arabidopsis thaliana*)	–	No	*bar* (*Streptomyces hygroscopicus*)	*3′g7* (*Agrobacterium tumefaciens*)
	Pta29 (*Nicotiana tabacum*)	–	No	*barnase* (*Bacillus amyloliquefaciens*)	*nos* (*A. tumefaciens*)
RF3	*PssuAt* (*A. thaliana*)	–	No	*bar* (*S. hygroscopicus*)	*3′g7* (*A. tumefaciens*)
	Pta29 (*N. tabacum*)	–	No	*barstar* (*B. amyloliquefaciens*)	*nos* (*A. tumefaciens*)

CTP: chloroplast transit peptide; UTR: untranslated region; –: when no element was specifically introduced to optimise expression.
*: Source of genetic information.

Table 4: Characteristics and intended effects of the events stacked in the three-event stack oilseed rape MON 88302 × MS8 × RF3

Event	Protein	Donor organism and biological function	Intended effects in GM plant
MON 88302	CP4 EPSPS	Based on a gene from *Agrobacterium* sp. strain CP4. 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) is an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Herrmann, 1995)	The bacterial CP4 EPSPS confers tolerance to glyphosate-containing herbicides, as it has a greatly reduced affinity towards glyphosate as compared to the plant endogenous enzyme

Event	Protein	Donor organism and biological function	Intended effects in GM plant
MS8	PAT	Based on a gene from *Streptomyces hygroscopicus*. Phosphinothricin-acetyl-transferase (PAT) confers resistance to the antibiotic bialaphos (Thompson et al., 1987)	Expression of PAT in oilseed rape MS8 confers tolerance to glufosinate-ammonium-containing herbicides
	Barnase	Based on a gene from *Bacillus amyloliquefaciens.* Barnase is a specific extracellular ribonuclease secreted by the bacterium	In MS8, the barnase coding sequence is under the control of a specific promoter (Pta29). It is only expressed in the tapetum cells during anther development, and results in male sterility
RF3	PAT	Based on a gene from *Streptomyces hygroscopicus*. Phosphinothricin-acetyl-transferase (PAT) confers resistance to the antibiotic bialaphos (Thompson et al., 1987)	Expression of PAT in oilseed rape RF3 confers tolerance to glufosinate-ammonium-containing herbicides
	Barstar	Based on a gene from *Bacillus amyloliquefaciens.* Barstar is a specific inhibitor of barnase; it protects the bacterium from the effects of barnase	In RF3, the barstar coding sequence is under the control of a specific promoter (Pta29). It is only expressed in the tapetum cells, and leads to restoration of fertility after crossing with oilseed rape MS8

3.3.1.2. Integrity of the events in the three-event stack oilseed rape MON 88302 × MS8 × RF3

The genetic stability of the inserted DNA over multiple generations in the three single oilseed rape events was demonstrated previously (Table 2). Integrity of the single events was demonstrated in the F_1 generation of the three-event stack oilseed rape by Southern analyses.[8]

3.3.1.3. Information on the expression of the inserts[9]

Plants were grown in five locations (four replicated plots) under field conditions in Chile and North America in 2011 and 2012.[10] The presence of the Barnase and Barstar proteins is limited to tapetum cells during anther development. Therefore, an analysis of their levels in other tissues was not considered relevant. The levels of CP4 EPSPS and PAT proteins in the three-event stack oilseed rape and the three single oilseed rape events were quantified by enzyme-linked immunosorbent assay (ELISA). Protein levels determined in seeds are reported and discussed below (Table 5). The levels of CP4 EPSPS and PAT in the three-event stack oilseed rape were compared to the corresponding levels in the single oilseed rape events.[10]

There are small differences between the levels of proteins in seeds (F_2 generation) produced by the three-event stack oilseed rape compared to the respective single oilseed rape events (Table 5). Such differences in expression levels between the single oilseed rape events and the three-event stack oilseed rape are not unexpected, and may be in part explained by the differences in zygosity of the transgenes between the single oilseed rape events and the three-event stack oilseed rape.

Table 5: Means, standard deviation and ranges of protein levels (μg/g dry weight) in seeds from oilseed rape MON 88302, MS8, RF3 and the three-event stack oilseed rape[(d)]

Protein	Protein levels in seeds			
	MON 88302 × MS8 × RF3	MON 88302	MS8	RF3
CP4 EPSPS	28.7[(a)] ± 2.6[(b)]	35.9 ± 3.8	–	–
	22.8–34.5[(c)]	30.2–43.6		

[8] Part II Scientific information, Section A2.2.2.
[9] Part II Scientific information, Section A2.2.3.
[10] Part II Scientific information, Section A2.2.3 – Annex: New (2013).

Protein	Protein levels in seeds			
	MON 88302 × MS8 × RF3	MON 88302	MS8	RF3
PAT	0.740 ± 0.15	–	0.324 ± 0.097	1.15 ± 0.18
	0.462–0.967		0.225–0.494	0.866–1.46

–: not assayed.
(a): Mean.
(b): Standard deviation.
(c): Range.
(d): As reported by the applicant.

3.3.1.4. Conclusions of the molecular characterisation

The molecular data establish that the events stacked in the three-event stack oilseed rape have retained their integrity. Protein expression analyses showed some difference between the levels in the single oilseed rape events and the three-event stack oilseed rape which are not unexpected. Therefore, there is no indication of interaction that may affect the integrity of the events and the levels of the newly expressed proteins in the three-event stack oilseed rape.

Based on known biological function of the newly expressed proteins, functional interaction between the Barnase and Barstar proteins are expected. These proteins are expressed in plant tissues (i.e. tapetum cells of the flower buds only) that are not present in food or feed derived from the three-event stack oilseed rape. No functional interaction is expected for the other newly expressed proteins.

Potential interactions are further assessed for their safety implications to human and animals in Section 3.3.3, and the environment in Section 3.3.4.

3.3.2. Comparative assessment

3.3.2.1. Choice of comparator and production of material for the comparative assessment[11]

Application EFSA-GMO-NL-2013-119 presents data on agronomic and phenotypic characteristics, as well as on seed composition of the three-event stack oilseed rape derived from field trials performed in Chile in 2011/2012 and North America (Canada and US) in 2012 (Table 6).

Table 6: Overview of comparative assessment studies with the three-event stack oilseed rape MON 88302 × MS8 × RF3 provided in the application EFSA-GMO-NL-2013-119

Study focus	Study details	Comparators	Commercial reference varieties
Agronomic and phenotypic characteristics, composition	2011/2012, Chile (2 sites) and 2012, North America (6 sites)	Conventional counterpart (Ebony)	15 non-GM oilseed rape varieties

Field trials for the agronomic/phenotypic and compositional characterisation of oilseed rape MON 88302 × MS8 × RF3 were performed at eight different locations in typical oilseed rape growing regions of Chile during the growing season 2011/2012 (two locations) and North America (Canada and US) during the growing season 2012 (six locations). At each site, the following materials were grown in a randomised complete block design with four replicates: oilseed rape MON 88302 × MS8 × RF3, the conventional counterpart and different non-GM oilseed rape reference varieties, all treated with required maintenance pesticides (including conventional herbicides); and oilseed rape MON 88302 × MS8 × RF3 treated with the intended herbicides, in addition to maintenance pesticides. A total of 15 non-GM oilseed rape reference varieties were included in the field trials (at least three per site). In these field trials, the comparator was a non-GM oilseed rape line (Ebony) with a genetic background similar to that of oilseed rape MON 88302 × MS8 × RF3 (as documented by the pedigree), and was therefore considered to be the appropriate conventional counterpart.

[11] Part II Scientific information, Section A3.1.

Statistical analysis of field trials data

The statistical analysis of the agronomic, phenotypic and compositional data from the 2011/2012 and 2012 field trials followed the recommendations of the GMO Panel (EFSA GMO Panel, 2010b, 2011a). This includes, for each of the two treatments of oilseed rape MON 88302 × MS8 × RF3, the application of a difference test (between the GM plant and its conventional counterpart) and an equivalence test (between the GM plant and the set of commercial non-GM oilseed rape reference varieties).[12]

3.3.2.2. Agronomic and phenotypic analysis[13]

Eleven agronomic and phenotypic endpoints were measured in the field trials (Section 3.3.2.1): early and final stand count, days-to-first-flowering, male fertility, plant height, seed maturity preharvest, lodging, pot shattering, seed moisture, seed quality and yield. Visually observable responses to naturally occurring diseases, abiotic stress and arthropod damage were also recorded in order to provide indications of altered stress responses of oilseed rape MON 88302 × MS8 × RF3 compared with its conventional counterpart.

Statistically significant differences between the three-event stack oilseed rape treated or untreated with the intended herbicides (in addition to maintenance pesticides) and its conventional counterpart were observed for: plant height, pod shattering, seed moisture and final stand count. For these endpoints, the test of equivalence indicated that the estimated means and confidence intervals for the three-event stack oilseed rape were within the equivalence limits from the non-GM oilseed rape reference varieties (equivalence category I; EFSA GMO Panel, 2011a).

The endpoint yield showed no statistically significant differences between the three-event stack oilseed rape treated with the intended herbicides and its conventional counterpart, and was more likely equivalent than not (equivalence category II).

There was no statistically significant difference between three-event stack oilseed rape treated or untreated with the intended herbicides and its conventional counterpart for the remaining agronomic and phenotypic endpoints. Additionally, no altered stress responses of three-event stack oilseed rape were observed compared with its conventional counterpart with regard to visually observable response to naturally occurring diseases, abiotic stress and arthropod damage.

3.3.2.3. Compositional analysis[14]

Oilseed rape seeds harvested from the field trials in Chile in 2011/2012 and North America in 2012 (Table 6) were analysed for 71 constituents,[15] including the key constituents recommended by the OECD (2011). For 10 fatty acids,[16] more than 50% of the observations were below the limit of quantification. The statistical analysis was applied to the remaining 61 constituents (endpoints).

For two endpoints (level of phosphorus and indolyl glucosinolates), equivalence could not be determined because of the very small variation among the non-GM oilseed rape reference varieties. Of the two endpoints, only phosphorus level in oilseed rape MON 88302 × MS8 × RF3 (treated) was found significantly different from the conventional counterpart. The estimated mean levels of phosphorus were 7.3 g/kg DM (oilseed rape MON 88302 × MS8 × RF3 treated with the intended herbicides; DM, dry matter) and 6.9 g/kg DM (conventional counterpart).

[12] The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence. In detail, the four outcomes are: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence).

[13] Part II Scientific information, Section A3.4; additional information: 21/9/2015 and 11/7/2016.

[14] Part II Scientific information, Section A3.3; additional information 21/9/2015 and 11/7/2016.

[15] The constituents included proximates and fibre (ash, moisture, protein, total fat, carbohydrates by calculation, crude fibre, acid detergent fibre (ADF) and neutral detergent fibre (NDF)), amino acids (alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine), fatty acids (caprylic (8:0), capric (10:0), lauric (12:0), myristic (14:0), myristoleic (14:1), pentadecanoic (15:0), pentadecenoic (15:1), palmitic (16:0), palmitoleic (16:1), heptadecanoic acid (17:0), heptadecenoic acid (17:1), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), γ-linolenic (18:3), arachidic (20:0), eicosenoic (20:1), eicosadienoic (20:2), eicosatrienoic (20:3), arachidonic (20:4), behenic (22:0), erucic (22:1), lignoceric (24:0) and nervonic (24:1)), vitamins (α-tocopherol and phylloquinone), minerals (calcium, chloride, copper, iron, magnesium, manganese, molybdenum, potassium, phosphorus, sodium, sulfur and zinc) and antinutrients (alkyl glucosinolates, indolyl glucosinolates, total glucosinolates, phytic acid, sinapine and total tannins).

[16] Caprylic acid (8:0), capric acid (10:0), lauric acid (12:0), myristoleic acid (14:1), pentadecanoic acid (15:0), pentadecenoic acid (15:1), γ-linolenic acid (18:3), eicosatrienoic acid (20:3), arachidonic acid (20:4) and erucic acid (22:1).

The combination of the test of difference and the test of equivalence could be applied to the remaining 59 endpoints, with the following results:

- For oilseed rape MON 88302 × MS8 × RF3 (not treated), statistically significant differences with the conventional counterpart were identified for 28 endpoints.[17] All the endpoints fell under equivalence category I or II.
- For oilseed rape MON 88302 × MS8 × RF3 (treated), statistically significant differences with the conventional counterpart were identified for 13 endpoints.[18] All the endpoints fell under equivalence category I or II.

The GMO Panel assessed all the compositional differences between oilseed rape MON 88302 × MS8 × RF3 and its conventional counterpart. After considering the well-known biological role of the compounds, the outcome of the equivalence test, and the magnitude of the changes observed, the GMO Panel did not identify any need for further food/feed safety assessment.

3.3.2.4. Conclusions of the comparative assessment

The GMO Panel concludes that none of the differences identified in seed composition and in the agronomic and phenotypic characteristics tested between oilseed rape MON 88302 × MS8 × RF3 and its conventional counterpart needs further assessment regarding food and feed safety.

Moreover, none of the differences identified in the agronomic and phenotypic characteristics tested between oilseed rape MON 88302 × MS8 × RF3 and its conventional counterpart needs further assessment regarding its potential environmental impact.

3.3.3. Food and feed safety assessment

3.3.3.1. Effects of processing[19]

Based on the outcome of the comparative assessment, processing of oilseed rape MON 88302 × MS8 × RF3 into food and feed products is not expected to result in products being different from those of commercial non-GM oilseed rape varieties.

3.3.3.2. Toxicology[20]

Toxicological assessment of newly expressed proteins[21]

Four proteins (Barnase, Barstar, CP4 EPSPS and PAT) are newly expressed in oilseed rape MON 88302 × MS8 × RF3 (Table 4).

The expression of the Barnase and Barstar proteins is limited to a plant tissue which is not relevant as food and feed (tapetum cells during anther development, Section 3). Therefore, these proteins are not considered relevant for the food and feed safety assessment.

The GMO Panel has previously assessed the safety of the CP4 EPSPS and PAT proteins individually in the context of the single oilseed rape events, and no safety concerns were identified for humans and animals (Table 2). The GMO Panel is not aware of any new information that would change this conclusion.

The potential for a functional interaction between the newly expressed proteins CP4 EPSPS and PAT in oilseed rape MON 88302 × MS8 × RF3 has been assessed with regard to human and animal health. The two proteins are enzymes that catalyse distinct biochemical reactions and act on unrelated substrates in the plant. On the basis of the known biological function of the individual newly expressed proteins (Table 4), there is currently no expectation for possible interactions between these two proteins relevant for the food and feed safety. Since the individual proteins are considered safe for humans and animals, the same conclusion can be extended to their presence in the three-event stack oilseed rape.

[17] The constituents with significantly different levels were: protein, carbohydrates by calculation, crude fibre, ADF, NDF, alanine, arginine, aspartic acid, glycine, histidine, isoleucine, leucine, phenylalanine, serine, threonine, tryptophan, valine, myristic acid (14:0), palmitoleic acid (16:0), heptadecenoic acid (17:1), lignoceric acid (17:1), α-tocopherol, copper, iron, manganese, molybdenum, zinc and total tannins.

[18] The constituents with significantly different levels were: ash, crude fibre, myristic acid (14:0), palmitoleic acid (16:1), eicosenoic acid (20:1), α-tocopherol, manganese, molybdenum, zinc, sinapine, total tannins and phytic acid.

[19] Part II Scientific information, Section A3.5.

[20] Part II Scientific information, Section A4.

[21] Part II Scientific information, Section A4.2.

The GMO Panel concludes that there are no safety concerns to human and animal health related to the newly expressed proteins CP4 EPSPS and PAT in the three-event stack oilseed rape.

Toxicological assessment of components other than newly expressed proteins[22]

The three-event stack oilseed rape did not show any compositional differences to its conventional counterpart that would require further assessment (Section 3.3.2). No further food and feed safety assessment of components other than newly expressed proteins is therefore required.

3.3.3.3. Animal studies with the food/feed derived from GM plants[23]

No animal studies with food/feed derived from oilseed rape MON 88302 × MS8 × RF3 were provided by the applicant (e.g. 90-day toxicity feeding studies in rodents or feeding studies in young rapidly growing animal species).

No substantial modifications in the composition of the three-event stack oilseed rape, no indication for potential occurrence of unintended effects based on the preceding molecular, compositional or phenotypic analyses, and no indication of possible interactions between the events were identified (Sections 3.3.1 and 3.3.2). Therefore, no animal studies on the food and feed derived from the three-event stack oilseed rape are required (EFSA GMO Panel, 2011a).

3.3.3.4. Allergenicity[24]

For allergenicity assessment, a weight-of-evidence approach was followed, taking into account all of the information on the newly expressed proteins, since no single piece of information or experimental method yields sufficient evidence to predict allergenicity (Codex Alimentarius, 2009; EFSA GMO Panel, 2011a). In addition, when known functional aspects of the newly expressed protein or structural similarity to known adjuvants may indicate an adjuvant activity, the possible role of these proteins as adjuvants is considered. When newly expressed proteins with a potential adjuvant activity are expressed together, possible interactions increasing adjuvanticity and impacting the allergenicity of the GM crop are assessed.

Assessment of allergenicity of the newly expressed proteins[25]

The GMO Panel has previously evaluated the safety of the CP4 EPSPS and PAT proteins, and no concerns on allergenicity were identified in those applications (Table 2). No new information on allergenicity of the newly expressed CP4 EPSPS and PAT proteins that might change the previous conclusions of the GMO Panel has become available. Based on current knowledge, and as none of the newly expressed proteins showed allergenicity, no reasons for concern regarding the simultaneous presence of these newly expressed proteins in this three-event stack oilseed rape were identified.

No information available on the structure or function of the newly expressed CP4 EPSPS and PAT proteins would suggest an adjuvant effect of the individual proteins, or of their simultaneous presence in the three-event stack oilseed rape, resulting in or increasing an eventual immunoglobulin E (IgE) response to a bystander protein.

Assessment of allergenicity of GM plant products[26]

The GMO Panel regularly reviews the available publications on food allergy to oilseed rape. However, to date, oilseed rape has not been considered a common allergenic food (OECD, 2011).[27] Therefore, the GMO Panel did not request experimental data to analyse the allergen repertoire of GM oilseed rape.

In the context of this application and considering the data from the molecular characterisation, the compositional analysis and the assessment of the newly expressed proteins (Sections 3.3.2 and 3.3.3.2), the GMO Panel identified no indications of a potentially increased allergenicity of food and feed derived from the three-event stack oilseed rape compared to that derived from its conventional counterpart.

[22] Part II Scientific information, Section A4.3 and A4.4.
[23] Part II Scientific information, Section A4.5.
[24] Part II Scientific information, Section A5.
[25] Part II Scientific information, Section A5.1 and additional information 27/9/2016.
[26] Part II Scientific information, Section A5.2.
[27] Directive 2007/68/EC of the European Parliament and of the Council of 27 November 2007 amending Annex IIIa to Directive 2000/13/EC of the European Parliament and of the Council as regards certain food ingredients. OJ L 310, 27.11.2007, p. 11–14.

3.3.3.5. Nutritional assessment of GM food/feed[28]

The intended trait of oilseed rape MON 88302 × MS8 × RF3 is herbicide tolerance, with no intention to alter nutritional parameters. Comparison of the composition of oilseed rape MON 88302 × MS8 × RF3 with its conventional counterpart and reference varieties did not identify differences that would require further safety assessment. From these data, an impact on the nutritional value of food and feed derived from the three-event stack oilseed rape is not expected.

3.3.3.6. Conclusion of the food and feed safety assessment

The CP4 EPSPS and PAT proteins newly expressed in the three-event stack oilseed rape do not raise safety concerns for human and animal health. No interactions between these proteins relevant for food and feed safety of three-event stack oilseed rape were identified. Similarly, the GMO Panel did not identify indications of safety concerns regarding allergenicity or adjuvanticity related to the presence of the newly expressed proteins in oilseed rape MON 88302 × MS8 × RF3, or regarding the overall allergenicity of this three-event stack oilseed rape. The three-event stack oilseed rape is expected to be as nutritious as its conventional counterpart and the tested non-GM oilseed rape commercial reference varieties.

3.3.4. Environmental risk assessment[29]

Considering the scope of the application EFSA-GMO-NL-2013-119 (which excludes cultivation), the ERA of the three-event stack oilseed rape is concerned mainly with: (1) the exposure of bacteria to recombinant DNA in the gastrointestinal tract of animals fed GM material and bacteria present in environments exposed to faecal material of these animals (manure and faeces); and (2) accidental release into the environment of imported viable seeds from the three-event stack oilseed rape during transportation and processing.

3.3.4.1. Persistence and invasiveness of the GM plant[30]

Oilseed rape (*Brassica napus* AACC) is an allotetraploid species ($2n = 38$, genome constitution AACC), which has probably evolved through hybridisation and polyploidisation between the two diploid species *Brassica rapa* ($2n = 20$, AA) and *Brassica oleracea* ($2n = 18$, CC). It is an annual plant developed for agricultural production.

Survival of oilseed rape outside cultivation areas is possible. Demographic studies and surveys have shown the ability of oilseed rape (*B. napus*) to establish self-perpetuating populations outside agricultural areas, mainly in seminatural and ruderal habitats in different countries (e.g. Devos et al., 2012; Bauer-Panskus et al., 2013; COGEM, 2013; Hecht et al., 2014; Schulze et al., 2014; Katsuta et al., 2015; Bailleul et al., 2016; Busi and Powles, 2016; Franzaring et al., 2016; Nishizawa et al., 2016; Pandolfo et al., 2016). Oilseed rape is generally regarded as an opportunistic species, which can take advantage of disturbed sites (e.g. mowed areas) to germinate and capture resources rapidly. In undisturbed natural habitats, oilseed rape lacks the ability to establish stable populations over successive years, possibly due to the absence of competition-free germination sites (Crawley et al., 1993, 2001) and exposure to biological and abiotic stressors likely limiting fitness (COGEM, 2013; Busi and Powles, 2016). Once established in competition-free germination sites, feral populations decline over a period of years (Crawley and Brown, 1995, 2004; Knispel et al., 2008; Squire et al., 2011; Banks, 2014; Busi and Powles, 2016). However, if habitats are disturbed on a regular basis, then feral populations can persist for longer periods (Claessen et al., 2005a,b; Garnier et al., 2006). The persistence or recurrence of a population in one location is variously attributed to replenishment with fresh seed spills, to recruitment from seed emerging from the soil seedbank or shed by resident feral adult plants, or to redistribution of feral seed from one location to another (Pivard et al., 2008a,b; Bailleul et al., 2016).

The three-event stack oilseed rape has been developed for tolerance to glufosinate-ammonium- and glyphosate-containing herbicides. The combination of *pat* and CP4 *epsps* genes coding for herbicide tolerance traits can provide a potential agronomic and selective advantage to oilseed rape MON 88302 × MS8 × RF3 plants when exposed to glufosinate-ammonium- and/or glyphosate-containing herbicides.

[28] Part II Scientific information, Section A6.
[29] Part II Scientific information: Section E.
[30] Part II Scientific information: Sections E2 and E3.3.1 and Appendix 5.

The applicant presented agronomic and phenotypic data on the three-event stack oilseed rape gathered from field trials conducted in oilseed rape growing areas of Chile during the growing season 2011/2012 (two locations) and North America (Canada and US) during the growing season 2012 (six locations) (see Section 3.3.2.2). The data set showed no differences in phenotypic plant characteristics that indicate altered fitness, persistence and invasiveness of oilseed rape MON 88302 × MS8 × RF3 plants. It is therefore unlikely that oilseed rape MON 88302 × MS8 × RF3 plants will have a selective advantage from the genetic modification, except when they are exposed to glufosinate-ammonium- and/or glyphosate-containing herbicides.

No specific data were provided to compare seed dormancy of oilseed rape MON 88302 × MS8 × RF3 plants with its conventional counterpart. However, there is no evidence that tolerance to the herbicidal active substances glufosinate-ammonium or glyphosate would alter seed dormancy of GM herbicide-tolerant oilseed rape plants, compared to their appropriate comparators. Seed dormancy is more likely to be affected by the genetic background of parental genotypes than the acquisition of herbicide tolerance traits.

Since the general characteristics of the three-event stack oilseed rape remain unchanged compared to its conventional counterpart, its ability to establish feral populations mostly in ruderal habitats will remain. Seed import spills can therefore lead to the occurrence of feral oilseed rape MON 88302 × MS8 × RF3 plants, but these are unlikely to establish stable populations over time (reviewed by Devos et al., 2012). Should these plants be exposed to glufosinate-ammonium- and/or glyphosate-containing herbicides, they are likely to exhibit a selective advantage that could increase their occurrence locally (Londo et al., 2010, 2011; Watrud et al., 2011). However, the likelihood of such an event will be restricted to herbicide-treated areas with little biodiversity, so that environmental impacts will be minimal.

Overall, the occurrence of feral oilseed rape MON 88302 × MS8 × RF3 plants resulting from seed import spills is likely to be low under import conditions, and their occurrence would be confined mostly to ruderal habitats. These plants will therefore not create additional agronomic or environmental impacts compared to their conventional counterparts.

3.3.4.2. Potential for gene transfer

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through HGT of DNA, or through vertical gene flow via the dispersal of pollen from feral plants originating from spilled seeds.

Plant-to-bacteria gene transfer[31]

The potential for HGT of the recombinant DNA of the single oilseed rape events MON 88302, MS8 and RF3, and the two-event stack oilseed rape MS8 × RF3 was assessed previously by the GMO Panel (EFSA, 2005, 2009; EFSA GMO Panel, 2010c, 2012, 2014). No concern as a result of an unlikely, but theoretically possible, HGT of the recombinant genes to bacteria in the gut or other receiving environments was identified.

Analysis of the data on the origin of the inserted sequences was performed to further characterise the possibility of HGT by homologous recombination (see also Section 3.2). In the single oilseed rape event MON 88302, no elements of bacterial origin with sufficient length and identity to support homologous recombination were identified. The highest sequence identity of the DNA encoding for the codon optimised CP4 EPSPS protein to the DNA of native prokaryotic sequences was 84%, which triggers no further consideration of homology-facilitated recombination and gene replacement. In the oilseed rape events MS8 and RF3, three elements of bacterial origin with sufficient length and sequence identity to facilitate homologous recombination with native bacterial genes were identified: the 3′ untranslated region of gene 7 (length: 306 bp) from the octopine T_i plasmid of *Agrobacterium tumefaciens*; the *bar* gene (552 bp) of *Streptomyces hygroscopicus*; and the 3′ untranslated region of the *nopaline synthase* gene (*nos*) (261bp) of *A. tumefaciens*. The 3′ untranslated region of the barnase gene (114 bp) and the *barstar* gene (273 bp) both with sequence identity to native sequences from *Bacillus amyloliquefaciens* were detected in the oilseed rape event MS8 and RF3, respectively.

No pairs of sequences which would facilitate transfer of inserts by double homologous recombination were identified. The data revealed that the two sequences with sequence identity to *A. tumefaciens*, i.e., the gene 7 fragment and the T-nos 3′ untranslated regions are not located on different Ti-plasmids (octopine and nopaline type); thus, indicating no potential for facilitated double

[31] Part II Scientific information: Section E3.3.2.

homologous recombination of the *bar* gene to *A. tumefaciens*. All other genes with sequence identity to native bacterial genes would only facilitate recombination with their respective counterparts and thus could only result in gene replacement, and thus, not in the acquisition of a new trait for the possible recipients.

Synergistic effects of the recombinant genes in increasing the likelihood for HGT, for instance combinations of recombinogenic sequences, have not been identified. Since the three-event stack oilseed rape is produced from conventional crossing, close linkage of the different events is extremely unlikely due to the distances separating them within the plant genome. Therefore, the GMO Panel concludes that, in the context of the scope of the application EFSA-GMO-NL-2013-119, the unlikely, but theoretically possible, horizontal transfer of recombinant genes from this three-event stack oilseed rape to bacteria does not raise any environmental safety concern.

The identified genes of bacterial origin of oilseed rape MON 88302 × MS8 × RF3 are all ubiquitous in nature and are not expected to confer a selective advantage by gene replacement. Therefore, the GMO Panel concludes that the recombinant DNA in oilseed rape MON 88302 × MS8 × RF3 does not represent an environmental risk in relation to its potential for horizontal transfer to bacteria.

Plant-to plant-gene transfer[32]

Considering the scope of application EFSA-GMO-NL-2013-119 and the biology of oilseed rape, the potential of occasional feral GM oilseed rape plants originating from seed import spills to transfer recombinant DNA to sexually cross-compatible plants and the environmental consequences thereof were considered. As pointed out previously, the accidental spillage of imported oilseed rape seeds can result in the occurrence of feral plants often in ruderal and disturbed habitats, where they can survive and reproduce.

Oilseed rape is an open pollinating crop plant capable of cross-pollinating with other *Brassica* crops (Eastham and Sweet, 2002). If established adjacent to cross-compatible field crops, then feral oilseed rape MON 88302 × MS8 × RF3 plants arising from spilled seeds could pollinate oilseed rape crop plants. Shed seed from cross-pollinated crop plants could emerge as GM volunteers in subsequent crops, though the likelihood of this happening is extremely low under an import scenario (Squire et al., 2011; Devos et al., 2012).

Oilseed rape can also spontaneously hybridise with sexually compatible wild relatives. Several oilseed rape × wild relative hybrids have been reported in the scientific literature, but under field conditions transgene introgression has only been confirmed for progeny of oilseed rape × *B. rapa* hybrids (reviewed by Ellstrand et al., 1999, 2013; FitzJohn et al., 2007; Devos et al., 2009). For transgene introgression to occur, feral GM oilseed rape must require some overlap in flowering in time and space with compatible relatives. Subsequently, transgenes must be transmitted through successive backcross generations or selfing, so that they become stabilised into the genome of the recipient (de Jong and Rong, 2013; Garnier et al., 2014). Because of these barriers (Luijten et al., 2015), reported incidences of hybrids and backcrosses with *B. rapa* were therefore found to be low in fields (Jørgensen et al., 2004; Norris et al., 2004; Warwick et al., 2008; Elling et al., 2009), or at ports, along roadsides, and riverbanks (Saji et al., 2005; Aono et al., 2006, 2011; Yoshimura et al., 2006; Elling et al., 2009; Katsuta et al., 2015; Luijten et al., 2015).

The GMO Panel does not consider the occurrence of occasional feral oilseed rape MON 88302 × MS8 × RF3 plants, pollen dispersal and consequent cross-pollination as environmental harm in itself, as there is no evidence that the herbicide tolerance traits will enhance the vertical gene flow potential, or fitness, persistence or invasiveness of feral oilseed rape MON 88302 × MS8 × RF3, or cross-compatible plants such as hybridising wild relatives. However, when exposed to glufosinate-ammonium- and/or glyphosate-containing herbicides, occasional cross-compatible plants that acquired the herbicide tolerance traits through vertical gene flow are likely to exhibit a selective advantage, which may lead to their increased occurrence. The likelihood of such an event to happen will be restricted to herbicide-treated areas, so that environmental impacts will be minimal. Therefore, the GMO Panel considers that the acquisition of the herbicide tolerance traits by cross-compatible plants would not create additional agronomic or environmental impacts.

In conclusion, the GMO Panel considers that the likelihood of environmental effects as a consequence of the spread of genes from the three-event stack oilseed rape in Europe will not differ from that of conventional oilseed rape varieties, even after exposure to glufosinate-ammonium- and/or glyphosate-containing herbicides.

[32] Part II Scientific information Section E3.3.1 and Appendix 5.

3.3.4.3. Interactions of the GM plant with target organisms[33]

Interactions occasional feral oilseed rape MON 88302 × MS8 × RF3 plants arising from seed import spills with target organisms are not considered to be a relevant issue by the GMO Panel, as there are no target organisms.

3.3.4.4. Interactions of the GM plant with non-target organisms[34]

Considering the scope of application EFSA-GMO-NL-2013-119, and the low level of exposure to the environment, potential interactions of occasional feral oilseed rape MON 88302 × MS8 × RF3 plants arising from seed import spills with non-target organisms are not considered to be a relevant issue by the GMO Panel.

3.3.4.5. Interactions with the abiotic environment and biogeochemical cycles[35]

Considering the scope of application EFSA-GMO-NL-2013-119, and the low level of exposure to the environment, potential interactions with the abiotic environment and biogeochemical cycles are not considered to be a relevant issue by the GMO Panel.

3.3.4.6. Conclusion of the environmental risk assessment

In the case of accidental release into the environment of viable seeds of the three-event stack oilseed rape, there are no indications of an increased likelihood of establishment and spread of feral oilseed rape MON 88302 × MS8 × RF3 plants, or hybridising wild relatives, unless these plants are exposed to glufosinate-ammonium- and/or glyphosate-containing herbicides. However, this will not result in different environmental impacts compared to conventional oilseed rape. Considering the scope of application EFSA-GMO-NL-2013-119, interactions with the biotic and abiotic environment were not considered to be relevant issues. Risks associated with an unlikely but theoretically possible HGT of recombinant DNA from the three-event stack oilseed rape to bacteria have not been identified.

Considering the novel combination of events, the introduced traits, the outcome of the comparative analysis, the routes of exposure and the limited exposure levels, the GMO Panel concludes that oilseed rape MON 88302 × MS8 × RF3 would not raise environmental safety concerns in the event of accidental release of viable GM oilseed rape seeds into the environment.

3.3.5. Conclusion on the three-event stack oilseed rape MON 88302 × MS8 × RF3

No new data on the single oilseed rape events MON 88302, MS8 and RF3 leading to a modification of the original conclusions on their safety were identified.

The combination of oilseed rape events MON 88302, MS8 and RF3 in the three-event stack oilseed rape did not give rise to issues pertaining to the molecular, agronomic/phenotypic or compositional characteristics of the three-event stack oilseed rape that would require further investigation in terms of food and feed safety and nutrition.

The newly expressed proteins in the three-event stack oilseed rape do not raise safety concerns for human and animal health and the environment in light of the scope of this application.

No indications of interactions between the events based on the biological functions of the newly expressed proteins that would raise a safety issue were identified in oilseed rape MON 88302 × MS8 × RF3. Comparison of the levels of the newly expressed proteins between the three-event stack oilseed rape and those of the single oilseed rape events did not reveal an interaction at protein expression level.

Considering the introduced traits and the outcome of the comparative analysis, the routes of exposure and limited exposure levels, the GMO Panel concludes that the three-event stack oilseed rape MON 88302 × MS8 × RF3 would not raise safety concerns in the event of accidental release of viable GM oilseed rape seeds into the environment.

No scientific information that could change the conclusions on this three-event stack was retrieved in a literature search covering the period since the time of validity of the application.[36] The GMO

[33] Part II Scientific information Section E3.3.
[34] Part II Scientific information Section E3.4.
[35] Part II Scientific information Section E3.6.
[36] Additional information 27/9/2016.

Panel concludes that the three-event stack oilseed rape is as safe and as nutritious as its conventional counterpart in the context of its scope.

3.4. Risk assessment of the subcombinations

The GMO Panel guidance establishes the principle that where all single events have been assessed, the risk assessment of stacked events focuses on issues related to: (a) stability of the events; (b) expression of the events; and (c) potential interactions between the events (EFSA GMO Panel, 2011a).

For those subcombinations for which no specific data have been submitted and which have not been previously assessed by the GMO Panel (Table 1), the risk assessment takes as its starting point the assessment of the single oilseed rape events, and uses the data generated for the three-event stack, as well as all the additional data available on subcombinations previously assessed by the GMO Panel.

3.4.1. Subcombination previously assessed

The two-event stack oilseed rape MS8 × RF3 has been assessed previously by the GMO Panel, and no safety concerns were identified (EFSA GMO Panel, 2012, 2009). A literature search revealed no new scientific information relevant to the risk assessment of this two-event stack oilseed rape that became available since the validation of the application EFSA-GMO-NL-2013-119.[36] Consequently, the GMO Panel considers that its previous conclusions on this subcombination remain valid.

3.4.2. Subcombinations not previously assessed

The two-event stacks oilseed rape MON 88302 × MS8 and MON 88302 × RF3 have not been previously assessed by the GMO Panel, and no experimental data specific for these two subcombinations were provided in this application. A literature search revealed no scientific information relevant to the risk assessment of the two-event stacks oilseed rape that became available since the validation of the application EFSA-GMO-NL-2013-119.[36]

3.4.2.1. Stability of the events

The genetic stability of the inserted DNA over multiple generations in the three single oilseed rape events was demonstrated previously (Table 2). Integrity of the events was demonstrated in the three-event stack MON 88302 × MS8 × RF3 (Section 3.3.1) and in the two-event stack MS8 × RF3 (Table 2). The GMO Panel therefore finds no reasons to expect the loss of integrity of the events in the subcombinations MON 88302 × MS8 and MON 88302 × RF3.

3.4.2.2. Expression of the events

The GMO Panel assessed whether the combination of any of the three events by conventional crossing could result in significant changes in expression levels of the newly expressed proteins, as this could indicate an unexpected interaction between the events. Based on the current knowledge on the molecular elements introduced, there is no reason to expect interactions that would affect the levels of the newly expressed proteins in these subcombinations compared with those in the single oilseed rape events. This assumption was confirmed by comparing the levels of the newly expressed proteins of each single oilseed rape event with those of the three-event stack oilseed rape: the small differences in levels observed between the single oilseed rape events and the three-event stack oilseed rape were not unexpected (Section 3.3.1.3), revealing no interaction manifesting at protein expression level. In addition, expression data from the two-stack oilseed rape MS8 × RF3 were similar to those observed in each of the single oilseed rape events (Table 2), thereby confirming that interactions affecting expression levels of the newly expressed proteins in a way that require further assessment are not expected in oilseed rape MON 88302 × MS8 and MON 88302 × RF3.

3.4.2.3. Potential interactions between the events

The GMO Panel assessed the potential interactions between events, due to their combination in the two-event stacks oilseed rape, taking into consideration intended traits and unintended effects.

Based on the known biological functions of the individual newly expressed proteins (Table 4), there is currently no expectation for possible functional interactions between these proteins in oilseed rape MON 88302 × MS8 and MON 88302 × RF3 relevant for the food and feed and environment safety.

A potential unintended effect was suggested for days-to-first flowering in oilseed rape MON 88302 (EFSA GMO Panel, 2014). Although the difference in days-to-first flowering was not observed in oilseed rape MON 88302 × MS8 × RF3 and did not raise safety concerns for oilseed rape MON 88302, the GMO Panel considered whether this could interact with the intended traits expressed in the oilseed rape MON 88302 × MS8 and MON 88302 × RF3. The GMO Panel is of the opinion that no interactions between the intended traits and the delay in days-to-first flowering observed in oilseed rape MON 88302 (EFSA GMO Panel, 2014) can be hypothesised for the subcombinations MON 88302 × MS8 and MON 88302 × RF3.

3.4.3. Conclusion

Since no new safety concerns were identified for the previously assessed two-event stack oilseed rape MS8 × RF3, the GMO Panel considers that its previous conclusions on this subcombination remain valid. For the two subcombinations MON 88302 × MS8 and MON 88302 × RF3 for which no experimental data have been provided, the GMO Panel assessed possible interactions between the events, and concludes that different combinations of the events MON 88302, MS8 and RF3 would not raise safety concerns in these subcombinations. These two subcombinations are therefore expected to be as safe as the single oilseed rape events, the previously assessed two-event oilseed rape stack MS8 × RF3, and three-event stack oilseed rape MON 88302 × MS8 × RF3.

3.5. Post-market monitoring

3.5.1. Post-market monitoring of GM food/feed[37]

No relevant compositional, agronomic and phenotypic changes were identified in three-event stack oilseed rape MON 88302 × MS8 × RF3 when compared with its conventional counterpart. Furthermore, the overall intake or exposure is not expected to change because of the introduction of oilseed rape MON 88302 × MS8 × RF3 into the market. The two-event stack oilseed rape MS8 × RF3 has been previously assessed and no safety concerns were identified and oilseed rape MON 88302 × MS8 and MON 88302 × RF3 subcombinations are expected to be as safe as the single oilseed rape events, the previously assessed two-event oilseed rape stack MS8 × RF3, and three-event stack oilseed rape MON 88302 × MS8 × RF3. Therefore, the GMO Panel considers that the post-market monitoring of oilseed rape MON 88302 × MS8 × RF3 and its subcombinations is not necessary.

3.5.2. Post-market environmental monitoring[38]

The objectives of a PMEM plan according to Annex VII of Directive 2001/18/EC are: (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the ERA are correct; and (2) 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 ERA.

Monitoring is related to risk management, and thus a final adoption of the PMEM plan falls outside the mandate of EFSA. However, the GMO Panel gives its opinion on the scientific content of the PMEM plan provided by the applicant (EFSA GMO Panel, 2011b).

As the ERA did not identify potential adverse environmental effects from the three-event stack oilseed rape and the already assessed two-event stack oilseed rape MS8 × RF3, no case-specific monitoring is required.

The PMEM plans proposed by the applicant for the three-event stack oilseed rape and the previously assessed two-event stack oilseed rape MS8 × RF3 (EFSA, 2005, 2009; EFSA GMO Panel, 2012) include: (1) 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; (2) a coordinating system newly established by EuropaBio for the collection of the information recorded by the various operators; and (3) the review of relevant studies/scientific publications retrieved from literature searches (Lecoq et al., 2007; Windels et al., 2008). The applicant proposes to submit a PMEM report on an annual basis.

[37] Part I Section D7.11.
[38] Part II Scientific information: Section E4.

The GMO Panel considers the scope of the PMEM plans provided by the applicant is consistent with the scope of the three-event stack oilseed rape and the already assessed two-event stack oilseed rape MS8 × RF3. The GMO Panel agrees with the reporting intervals proposed by the applicant in the PMEM plans. However, the PMEM plan submitted by the applicant for the three-event stack oilseed rape does not include any provisions for the two-two-event stacks not previously assessed by the GMO Panel. Therefore, the GMO Panel recommends the applicant to revise the plan accordingly.

In addition, the 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 MON 88302 × MS8 × RF3.

Should risk managers consider the control of feral oilseed rape plants desirable, then the implementation of appropriate communication means for the timely reporting of control failures of feral oilseed rape populations may be recommended.

4. Overall conclusions and recommendations

No new data on the single oilseed rape events MS8, RF3 and MON 88302 that would lead to a modification of the original conclusions on their safety were identified.

The combination of the events MON 88302, MS8 and RF3 in the three-event stack oilseed rape did not give rise to issues relating to molecular, agronomic/phenotypic and compositional characteristics regarding food and feed safety. The newly expressed proteins in the three-event stack oilseed rape did not raise concerns for human and animal health. The compositional data indicate that oilseed rape MON 88302 × MS8 × RF3 is expected to be as nutritious as its conventional counterpart and the tested non-GM oilseed rape commercial reference varieties. The GMO Panel considers that there is no reason to expect interactions that could impact on food and feed safety. Considering the introduced traits, the outcome of the comparative analysis, the routes of exposure and the limited exposure levels, the GMO Panel concludes that oilseed rape MON 88302 × MS8 × RF3 would not raise environmental safety concerns in the event of accidental release of viable GM oilseed rape seeds into the environment, irrespective of the possible interactions between the individual events within this three-stack oilseed rape.

The GMO Panel concludes that oilseed rape MON 88302 × MS8 × RF3 is as safe and as nutritious as its conventional counterpart and the tested non-GM oilseed rape reference varieties in the context of the scope of this application.

Since no new data on the previously assessed two-event stack oilseed rape MS8 × RF3 that would lead to a modification of the original conclusions on their safety were identified, the GMO Panel considers that its previous conclusions on this two-event stack oilseed rape remain valid. For the two subcombinations MON 88302 × MS8 and MON 88302 × RF3 for which no experimental data have been provided, the GMO Panel assessed possible interactions between the events, and concludes that different combinations of the events MON 88302, MS8 and RF3 would not raise safety concerns in these subcombinations. These two subcombinations are therefore expected to be as safe and as nutritious as the single oilseed rape events, the previously assessed two-event stack oilseed rape MS8 × RF3, and three-event stack oilseed rape MON 88302 × MS8 × RF3.

Given the absence of safety concerns identified on food and feed derived from the three-event stack oilseed rape MON 88302 × MS8 × RF3 and its subcombinations MS8 × RF3, MON 88302 × MS8, and MON 88302 × RF3, the GMO Panel considers that post-market monitoring of these products is not necessary.

The GMO Panel considers that the scope of the PMEM plans provided by the applicant is consistent with the scope of the three-event stack oilseed rape and the already assessed two-event stack oilseed rape MS8 × RF3. The GMO Panel agrees with the reporting intervals proposed by the applicant in the PMEM plans. However, the PMEM plan submitted by the applicant for the three-event stack oilseed rape does not include any provisions for two subcombinations that were not previously assessed. Therefore, the GMO Panel recommends the applicant to revise the plan accordingly.

Documentation as provided to EFSA

1) Letter from the Competent Authority of the Netherlands, received on 5 December 2013, concerning a request for placing on the market of genetically modified oilseed rape MON 88302 × MS8 × RF3 submitted jointly by Monsanto Company and Bayer CropScience in accordance with Regulation (EC) No 1829/2003 (application reference EFSA-GMO-NL-2013-119).

2) Acknowledgement letter 13 December 2013 from EFSA to the Competent Authority of the Netherlands.
3) Letter from EFSA to applicant dated 28 January 2014 requesting additional information under completeness check.
4) Letter from applicant to EFSA received on 17 March 2014 providing additional information under completeness check.
5) Letter from applicant to EFSA received on 31 March 2014 providing additional information under completeness check.
6) Letter from EFSA to applicant dated 24 April 2014 delivering the 'Statement of Validity' of application EFSA-GMO-NL-2013-119 for placing on the market of genetically modified oil seed rape MON 8832 × MS8 x RF3 submitted by Monsanto and Bayer CropScience in accordance with Regulation (EC) No 1829/2003.
7) Letter from EFSA to applicant dated 24 April 2014 stopping the clock due to single event (MON 88302 - application reference EFSA-GMO-BE-2011-101) not finalised.
8) Letter from EFSA to applicant dated 4 June 2014 re-starting the clock due to single event (MON 88302 - application reference EFSA-GMO-BE-2011-101) finalised.
9) Letter from EFSA to applicant dated 3 July 2014 requesting additional information and stopping the clock.
10) Letter from applicant to EFSA received on 1 September 2014 providing additional information.
11) Letter from EFSA to applicant dated 17 October 2014 requesting additional information and maintaining the clock stopped.
12) Letter from applicant to EFSA received on 15 December 2014 providing additional information.
13) Letter from EFSA to applicant dated 24 February 2015 re-starting the clock.
14) Letter from EFSA to applicant dated 23 June 2015 requesting additional information and stopping the clock.
15) Letter from applicant to EFSA received on 21 September 2015 providing additional information.
16) Letter from applicant to EFSA received on 10 February 2016 requesting clarifications.
17) Letter from EFSA to applicant dated 11 February 2016 requesting additional information and maintaining the clock stopped.
18) Letter from EFSA to applicant dated 16 February 2016 requesting additional information and maintaining the clock stopped.
19) Letter from applicant to EFSA received on 30 March 2016 providing additional information.
20) Letter from EFSA to applicant dated 5 April 2016 providing clarifications.
21) Letter from EFSA to applicant dated 5 April 2016 providing clarifications.
22) Letter from EFSA to applicant dated 8 July 2016 requesting additional information and maintaining the clock stopped.
23) Letter from applicant to EFSA received on 11 July 2016 providing additional information.
24) Letter from applicant to EFSA received on 27 September 2016 providing additional information.
25) Email from EFSA to applicant dated 28 September 2016 re-starting the clock from 27 September 2016.
26) Letter from applicant to EFSA received on 30 November 2016 requesting clarifications.
27) Letter from EFSA to applicant dated 6 December 2016 providing clarifications.
28) Letter from applicant to EFSA received on 13 December 2016 requesting clarifications and providing additional information spontaneously.
29) Letter from applicant to EFSA to applicant dated 30 January 2017 requesting clarifications.
30) Letter from EFSA to applicant received on 1 February 2017 providing clarifications.

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Abbreviations

ADF	acid detergent fibre
CTP	chloroplast transit peptide
ELISA	enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	environmental risk assessment
EST	expressed sequence tag
GM	genetically modified
GMO	genetically modified organism
GMO Panel	EFSA Panel on Genetically Modified Organisms
HGT	horizontal gene transfer
Ig	immunoglobulin
NDF	neutral detergent fibre
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
OSR	oilseed rape
PAT	phosphinothricin acetyltransferase
PMEM	post-market environmental monitoring
UTR	untranslated region