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Assessment of genetically modified oilseed rape MS11 for food and feed uses, import and processing, under Regulation (EC) No 1829/2003 (application EFSA-GMO-BE-2016-138)

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Abstract

Oilseed rape MS11 has been developed to confer male sterility and tolerance to glufosinate-ammonium-containing herbicides. Based on the information provided in the application and in line with the scope of application EFSA-GMO-BE-2016-138, the genetically modified organism (GMO) Panel concludes that the molecular characterisation data and bioinformatic analyses do not identify issues requiring food/feed safety assessment. None of the identified differences in the agronomic/phenotypic characteristics tested between oilseed rape MS11 and its conventional counterpart needs further assessment. No conclusions can be drawn for the compositional analysis due to the lack of an appropriate compositional data set. No toxicological or allergenicity concerns are identified for the Barnase, Barstar and PAT/bar proteins expressed in oilseed rape MS11. Owing to the incompleteness of the compositional analysis, the toxicological, allergenicity and nutritional assessment of oilseed rape MS11 cannot be completed. In the case of accidental release of viable oilseed rape MS11 seeds into the environment, oilseed rape MS11 would not raise environmental safety concerns. The post-market environmental monitoring plan and reporting intervals are in line with the scope of the application. Since oilseed rape MS11 is designed to be used only for the production of hybrid seed, it is not expected to be commercialised as a stand-alone product for food/feed uses. Thus, seeds harvested from oilseed rape MS11 are not expected to enter the food/feed chain, except accidentally. In this context, the GMO Panel notes that, oilseed rape MS11 would not pose risk to humans and animals, while the scale of environmental exposure will be substantially reduced compared to a stand-alone product.

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Summary

In the present scientific opinion, the scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA) (hereafter referred to as the 'GMO Panel') reports the outcome of the risk assessment of oilseed rape MS11 in line with the scope of the application EFSA-GMO-BE-2016-138 which is for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003. In delivering its scientific opinion, the GMO Panel took into account application EFSA-GMO-BE-2016-138, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications. The GMO Panel conducted the assessment of oilseed rape MS11 following the principles described in Regulation (EU) No 503/2013 and its applicable guidelines for the risk assessment of genetically modified (GM) plants.

Oilseed rape MS11 is part of a breeding system intended to produce: (1) fertile oilseed rape hybrid seed MS11×RF3 (production scenario 1); and (2) new oilseed rape MS11 seed to maintain the line (production scenario 2).

In line with the scope of the application EFSA-GMO-BE-2016-138, the GMO Panel concludes that:

- The molecular characterisation data establish that oilseed rape MS11 contains a single insert consisting of one copy of the *pat/bar*, *barnase* and *barstar* expression cassettes. Bioinformatics analyses of the sequences encoding the newly expressed proteins and other open reading frames present within the insert or spanning the junctions between the insert and genomic DNA do not indicate significant similarities to toxins and allergens. The stability of the inserted DNA and of the introduced herbicide tolerance trait was confirmed over several generations. The levels of the Barnase, Barstar and PAT/bar proteins were obtained and reported adequately. The information provided on the protein characterisation indicate that the plant- and *E. coli*-produced Barnase, Barstar and PAT/bar proteins are equivalent and the *E. coli*-produced proteins can be used in the safety studies.
- The characteristics of the introduced traits of oilseed rape MS11 challenge the comparative analysis to the extent that it is not possible to produce the materials and collect the data for the comparative analysis without deviating from the requirements laid down in Regulation (EU) No 503/2013. None of the differences between oilseed rape MS11 and its conventional counterpart identified in the agronomic and phenotypic characteristics tested under the specific theoretical cultivation scenarios needs further assessment. Because of the heterogeneous genetic background of the seeds produced from oilseed rape MS11 treated with the intended herbicide, the compositional data cannot be considered adequate for the comparative analysis. Hence, no conclusions can be drawn for the compositional analysis.
- No toxicological and allergenicity concerns are identified regarding the Barnase, Barstar and PAT/bar proteins as expressed in oilseed rape MS11. Owing to the incompleteness of the compositional analysis, the toxicological, allergenicity and nutritional assessment of oilseed rape MS11 cannot be completed.
- Oilseed rape MS11 would not raise safety concerns in the event of accidental release of viable GM oilseed rape seeds into the environment.
- The post-market environmental monitoring (PMEM) plan proposed by the applicant is in line with the scope of the application and agrees with its reporting intervals.
- Based on the relevant publications identified through the literature searches, no safety issues pertaining to the uses of oilseed rape MS11 are identified. In the context of PMEM, the applicant could further fine-tune future literature searches according to the GMO Panel recommendations.
- In conclusion, in the absence of an appropriate comparative assessment and considering the scope of application EFSA-GMO-BE-2016-138 as defined by the applicant (food and feed uses, import and processing), the food/feed assessment of oilseed rape MS11 cannot be completed. However, the GMO Panel concludes that the oilseed rape MS11 is unlikely to have any adverse effect on the environment in the context of the scope of the application.

The GMO Panel notes that the oilseed rape MS11 is designed to be used only for the production of hybrid seed in the frame of a dedicated breeding system and is thus not expected to be commercialised as a stand-alone product for food/feed uses. Therefore, seeds harvested from oilseed rape MS11 are not expected to enter the food/feed chain, except in the case of accidental presence in products coming from non-EU countries. In this context, the GMO Panel notes that oilseed rape MS11 would not pose risks to humans and animals, while the scale of environmental exposure will be

substantially reduced compared to a stand-alone product. On the other hand, the conclusions on the molecular characterisation data and toxicity and allergenicity of the Barnase, Barstar and PAT/bar proteins, as expressed in oilseed rape MS11 remain unchanged.

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1. Introduction

The scope of the application EFSA-GMO-BE-2016-138 is for food and feed uses, import and processing of oilseed rape MS11 and does not include cultivation in the European Union (EU).

Oilseed rape MS11 was developed to confer male sterility and tolerance to the herbicidal active substance glufosinate-ammonium.

Oilseed rape MS11 expresses the *barnase* gene encoding for a ribonuclease with cytotoxic activity. Expression of the *barnase* gene is restricted to the tapetum cells during anther development by a tissue-specific promoter leading to male sterility due to the lack of viable pollen. Oilseed rape MS11 also expresses the *barstar* gene under the weak *Pnos* constitutive promoter to produce the Barstar protein. The Barstar protein forms a stable protein–protein complex with Barnase and inhibits its activity. The prophylactic *barstar* gene is included to enhance transformation frequency by limiting the negative effects of leaky expression of the *barnase* gene in undifferentiated plant tissues. In tapetum cells of oilseed rape MS11 plants, the *barstar* expression is not sufficient to prevent the cytotoxic effects of the *barnase* gene leading to the expected male sterile phenotype, while in the other tissues the low-level expression of *barstar* plays no role.

Oilseed rape MS11 is part of a breeding system intended to produce: (1) fertile oilseed rape hybrid seed MS11×RF3 (production scenario 1, below); and (2) new oilseed rape MS11 seed to maintain the line (production scenario 2, below).

As MS11 is male sterile, it must be pollinated by a male fertile plant to produce seeds. Thus, considering the two production scenarios, the breeding system necessitates the use of three oilseed rape lines:

- Oilseed rape MS11. This line is male sterile (MS) and serves as a ‘mother’ plant that can set seed if pollinated by another oilseed rape plant during seed production.
- Oilseed rape RF3 (for scenario 1). This line is male fertile, serves as a pollen donor and restores fertility (RF) in the progeny of oilseed rape MS11 when crossed with oilseed rape MS11 during hybrid seed production. The RF3 line has a genetic background different from the one of MS11.
- A maintainer line (for scenario 2). This line serves to maintain the oilseed rape MS11 line over generations through the production of new oilseed rape MS11 seed. This line shares the same genetic background as oilseed rape MS11 but lacks the MS trait.

Seeds derived from oilseed rape MS11 crossed with a fertile oilseed rape line (e.g. maintainer line) will segregate 50:50 for the presence of event MS11. For this reason, a seed lot of oilseed rape MS11 is composed of a 50:50 mixture of seed containing/not containing the intended traits. Within the intended breeding system, MS11 seed lots are used in the two different production scenarios as follows:

- Scenario 1 (production of MS11×RF3). The MS11 seed lot is sown adjacent to the RF line, oilseed rape RF3. Upon treatment with glufosinate-ammonium containing herbicides, 50% of the MS11 plants are killed; the remaining 50% will be male sterile and herbicide tolerant. These MS11 plants will then be pollinated by the RF line. The resulting progeny (MS11×RF3 hybrid seeds) will be marketed for sowing: the seeds are expected to show hybrid vigour and superior yield (Wolko et al., 2019).
- Scenario 2 (production of new MS11). The MS11 seed lot is sown adjacent to a maintainer line. Upon treatment with glufosinate-ammonium containing herbicides, 50% of the MS11 plants are killed; the remaining 50% will be male sterile and herbicide tolerant. These MS11 plants will be then pollinated by the maintainer line. The resulting progeny (MS11 seeds) will be used to maintain the oilseed rape MS11 line for hybrid seed production (i.e. for scenario 1).

Based on the above-described breeding system, the GMO Panel notes that oilseed rape MS11 is intended to be used only for the production of hybrid seed; it is not intended to be commercialised as a stand-alone product for food and feed uses, import and processing. Hence, oilseed rape MS11 is not expected to enter the food and feed chain in the EU, except in the case of accidental presence in products coming from non-EU countries.

1.1. Background

On 16 December 2016, the European Food Safety Authority (EFSA) received from the Competent Authority of Belgium application EFSA-GMO-BE-2016-138 for authorisation of Oilseed rape MS11

(Unique Identifier BCS-BNØ12-7), submitted by Bayer CropScience (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003¹.

Following receipt of application EFSA-GMO-BE-2016-138, EFSA informed EU Member States and the European Commission, and made the application available to them. Simultaneously, EFSA published the summary of the application.²

EFSA checked the application for compliance with the relevant requirements of EFSA guidance documents, and, when needed, asked the applicant to supplement the initial application. On 08 March 2017, EFSA declared the application valid.

From validity date, EFSA and its scientific Panel on Genetically Modified Organisms (hereafter referred to as 'the GMO Panel') endeavoured to respect a time limit of 6 months to issue a scientific opinion on application EFSA-GMO-BE-2016-138. Such time limit was extended whenever EFSA and/or its GMO Panel requested supplementary information to the applicant. According to Regulation (EC) No 1829/2003, any supplementary information provided by the applicant during the risk assessment was made available to the EU Member States and European Commission (for further details, see the section 'Documentation', below).

In accordance with Regulation (EC) No 1829/2003, EFSA consulted the nominated risk assessment bodies of EU Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC³. The EU Member States had 3 months to make their opinion known on application EFSA-GMO-BE-2016-138 as of date of validity.

1.2. Terms of Reference as provided by the requestor

According to Articles 6 and 18 of Regulation (EC) No 1829/2003, EFSA and its GMO Panel were requested to carry out a scientific risk assessment of oilseed rape MS11 in the context of its scope as defined in application EFSA-GMO-BE-2016-138.

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).

The relevant information is made available in the EFSA Register of Questions including the information required under Annex II to the Cartagena Protocol; a labelling proposal; a Post-Market Environmental Monitoring (PMEM) plan as provided by the applicant; the method(s), validated by the Community reference laboratory, for detection, including sampling, identification of the transformation event in the food–feed and/or foods–feeds produced from it and the appropriate reference materials.²

2. Data and methodologies

2.1. Data

The GMO Panel based its scientific risk assessment of oilseed rape MS11 on the valid application EFSA-GMO-BE-2016-138, additional information provided by the applicant during the risk assessment, scientific comments submitted by EU Member States and relevant peer-reviewed scientific publications. In addition to this comprehensive information package, the GMO Panel also received unpublished studies submitted by the applicant in order to comply with the specific provisions of Regulation (EU) No 503/2013. A list of these additional unpublished studies is provided in Appendix A.

2.2. Methodologies

The GMO Panel conducted its assessment in line with the principles described in Regulation (EU) No 503/2013, its applicable guidelines (i.e. EFSA GMO Panel, 2010a,b, 2011a,b, 2015) and explanatory notes (i.e. EFSA, 2014, 2017a,b) for the risk assessment of GM plants. During its risk assessment, the GMO Panel also considered all additional unpublished studies as listed in Appendix A for potential effects on human and animal health and the environment.

¹ Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.

² Available online: <http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2016-00857>

³ 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.

For the 90-day animal feeding study, the GMO Panel took into account the criteria included in the 2011 EFSA Scientific Committee guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed (EFSA Scientific Committee, 2011) and the explanatory statement for its applicability (EFSA, 2014).

The GMO Panel also assessed the applicant's literature searches, which include a scoping review, in accordance with the recommendations on literature searching outlined in EFSA (2010, 2017a). In the frame of the contracts OC/EFSA/GMO/2013/01 and OC/EFSA/GMO/2014/01, contractors performed preparatory work and delivered reports on the methods applied by the applicant in performing bioinformatic and statistical analyses, respectively.

3. Assessment

3.1. Systematic literature review⁴

The GMO Panel assessed the applicant's literature searches on oilseed rape MS11, which include a scoping review, according to the guidelines given in EFSA (2010).

A systematic review as referred to in Regulation (EU) No 503/2013 was not provided in support to the risk assessment of application EFSA-GMO-BE-2016-138. Based on the outcome of the scoping review, the GMO Panel agrees that there is limited value of undertaking a systematic review for oilseed rape MS11 at present.

Although the overall quality of the performed literature searches is acceptable, the GMO Panel considers that future searches on oilseed rape MS11 could be further improved. The GMO Panel therefore recommends the applicant to use proximity operators that allow searching for terms that are adjacent in any order.

None of the relevant publications identified through the literature searches reported information pointing to safety issues associated with the intended uses of oilseed rape MS11.

3.2. Molecular characterisation

3.2.1. Transformation process and vector construct⁵

Oilseed rape MS11 was developed by *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*) mediated transformation of embryogenic callus derived from hypocotyl segments of oilseed rape (*Brassica napus*) variety N90-740 seeds with plasmid vector pTCO113. A helper Ti-plasmid pGV4000 was also used in the transformation process.

The plasmid pTCO113 used for the transformation contained three expression cassettes between the right and left borders of the T-DNA: *pat/bar*, *barnase* and *barstar*.

The *pat/bar* expression cassette contains the following genetic elements: the promoter region of the ribulose-1,5-biphosphate carboxylase small subunit gene from *Arabidopsis thaliana* (*PssuAt*), the coding sequence of the phosphinothricin acetyl transferase gene from *Streptomyces hygrosopicus* (*bar*) and the 3' untranslated region of the TL-DNA gene 7 from the *A. tumefaciens* octopine Ti plasmid (3'g7).

The *barnase* expression cassette contains the following genetic elements: the promoter of the anther-specific gene TA29 from *Nicotiana tabacum* (*Pta29*), the coding sequence and 3' untranslated region of the *barnase* gene from *Bacillus amyloliquefaciens* and 3' untranslated region of the nopaline synthase gene (3'nos) from the T-DNA of pTiT37. The *barstar* expression cassette contains the following genetic elements: the promoter region of the nopaline synthase (*Phos*) gene from *A. tumefaciens*, the coding sequence of the *barstar* gene from *Bacillus amyloliquefaciens*, and the 3' untranslated region of the TL-DNA gene 7 from the *A. tumefaciens* octopine Ti plasmid (3'g7).

The vector backbone contained elements necessary for the maintenance of the plasmid in bacteria and a fragment containing the *barstar* gene.

3.2.2. Transgene constructs in the GM plant⁶

Molecular characterisation of oilseed rape MS11 was performed by Southern analysis, polymerase chain reaction (PCR) and DNA sequence analysis, in order to determine insert copy number, size and

⁴ Dossier: Part II – Section 7; Additional information: 24/8/2018 and 14/6/2019.

⁵ Dossier: Part II – Section 1.2.1.

⁶ Dossier: Part II – Section 1.2.2.2; additional information 21/1/2020.

organisation of the inserted sequences and to confirm the absence of plasmid backbone sequences. The approach used was acceptable in terms of coverage and sensitivity.

Southern analyses indicated that oilseed rape MS11 contains a single insert, which consists of a single copy of the T-DNA in the same configuration as in the pTCO113. The insert and copy number were confirmed by multiple restriction enzyme/probe combinations covering the T-DNA region and flanking regions. PCR analyses confirmed the results obtained by the Southern analyses. The absence of vector backbone sequences was demonstrated by Southern analysis using backbone-specific overlapping probes.

The nucleotide sequence of the entire insert of oilseed rape MS11 together with 1,129 bp of the 5' and 1,302 bp of the 3' flanking regions was determined. The insert of 5,778 bp is identical to the T-DNA of pTCO113, except for the truncation of the border regions. A comparison of the flanking regions with the pre-insertion locus indicated that 40 bp of the parental genomic sequence had been deleted upon transformation. The possible interruption of known endogenous oilseed rape genes by the insertion in event MS11 was evaluated by bioinformatic analyses of the pre-insertion locus and of the genomic sequences flanking the insert. The results of these analyses did not reveal the interruption of any known endogenous gene in the oilseed rape MS11.

The results of segregation (see Section 3.2.5) and bioinformatic analyses established that the insert is located in the nuclear genome.

Updated bioinformatic analyses of the amino acid sequences of the newly expressed Barnase, Barstar and PAT/bar proteins revealed no significant similarities to known toxins and allergens. In addition, updated bioinformatic analyses of the newly created open reading frames (ORFs) within the insert or spanning the junctions between the insert and genomic DNA did not indicate significant similarities to toxins and allergens.

In order to assess the possibility for horizontal gene transfer by homologous recombination (HR), the applicant performed a sequence identity analysis of the regions of bacterial origin in oilseed rape MS11. The likelihood and potential consequences of plant to bacteria gene transfer are described in Section 3.5.1.2.

3.2.3. Protein characterisation and equivalence⁷

Oilseed rape MS11 expresses three new proteins Barnase, Barstar and PAT/bar.

Given the technical restraints in producing large enough quantities for safety testing from plants, these proteins were recombinantly produced in *Escherichia coli*. Prior to safety studies, a set of biochemical methods was employed to demonstrate the equivalence between oilseed rape and *E.coli*-produced proteins. Purified proteins from these two sources were characterised and compared in terms of their physico-chemical, structural and functional properties.

3.2.3.1. Barnase characterisation and equivalence

Considering the insert design, Barnase expression in oilseed rape MS11 is expected to be low and limited to only flower buds during anther development. Furthermore, based on the data submitted by the applicant, Barnase could not be detected by western blot analysis in crude extracts or immuno-affinity purified samples. Consequently, Barnase could not be extracted at sufficient quantity to experimentally demonstrate its equivalence with the *E.coli*-produced Barnase protein. Based on this information, a weight of evidence approach was employed by the applicant to demonstrate the equivalence between the plant and the *E. coli*-produced Barnase proteins. Amino acid sequence analysis of the *E. coli*-produced protein by mass spectrometry methods showed that it was 100% identical to the Barnase amino acid sequence deduced from the nucleotide sequence of the insert in oilseed rape MS11. Furthermore, the activity of the plant-derived Barnase protein was demonstrated *in vivo* by the male sterile phenotype exhibited by oilseed rape MS11 plants and the activity of the *E.coli*-produced protein was shown by a biochemical *in vitro* activity assay.

3.2.3.2. Barstar characterisation and equivalence

The expression of Barstar in oilseed rape MS11 is low; however, a concentrated crude protein extract from roots was used to carry out a western blot analysis. This analysis showed that plant and *E.coli*-produced Barstar proteins had the expected molecular weight of ~ 10 kDa and were comparably immunoreactive to Barstar protein-specific antibodies. Due to the low expression in oilseed rape MS11,

⁷ Dossier: Part II – Section 1.4.1.

Barstar could not be extracted in sufficient quantity to further experimentally demonstrate its equivalence with the *E. coli*-produced Barstar protein. Amino acid sequence analysis of the *E. coli*-produced Barstar by mass spectrometry methods showed that it was 100% identical to the Barstar amino acid sequence deduced from the nucleotide sequence of the insert in oilseed rape MS11. The function of Barstar in forming a protein–protein complex with Barnase is demonstrated *in vivo* by its prophylactic role in preventing the cytotoxic activity of Barnase in cells other than tapetum and *in vitro* by a study on the temperature-dependent Barnase–Barstar complex formation using the *E. coli*-produced protein.

3.2.3.3. PAT/bar characterisation and equivalence

SDS-PAGE and western blot analysis showed that plant and microbe-derived PAT/bar proteins had the expected molecular weight of ~ 20.5 kDa and were comparably immunoreactive to PAT/bar protein-specific antibodies. In addition, glycosylation detection analysis demonstrated that none of the PAT/bar proteins were glycosylated. Amino acid sequence analysis by mass spectrometry and N-terminal sequencing methods showed that both proteins showed that the protein matched the deduced sequence as defined by the *pat/bar* gene. These data also showed that the N-terminal methionine of the plant-derived PAT/bar protein was acetylated. Such modifications are common in eukaryotic proteins (e.g. Polevoda and Sherman, 2000) and have been previously assessed by the GMO Panel for proteins newly expressed in GM plants (e.g. EFSA GMO Panel, 2017a). Functional equivalence was demonstrated by a biochemical *in vitro* activity assay which showed that both proteins had comparable activity for the intended herbicide.

The protein characterisation data comparing the structural, biochemical and functional properties of plant and microbial PAT/bar proteins indicate that these proteins are equivalent. Barnase and Barstar expression in MS11 plants is low, and therefore, they could not be extracted at sufficient amounts to experimentally demonstrate their equivalence with the *E. coli*-produced proteins. Thus, a weight of evidence approach was employed to demonstrate the equivalence between the plant and the *E. coli*-produced Barnase and Barstar proteins. The GMO Panel considered the provided information sufficient and therefore accepts the use of the Barnase, Barstar and PAT/bar proteins produced in *E. coli* for the safety studies.

3.2.4. Information on the expression of the insert⁸

Protein levels of Barnase, Barstar and PAT/bar were analysed by enzyme-linked immunosorbent assays (ELISA) in material harvested in a field trial across three locations in the USA and Canada during the 2014 growing season. Samples analysed included whole plant (3–5 leaves, stem elongation and first flowering), root (stem elongation and first flowering), raceme (first flowering) and seed (maturity) both those treated and non-treated with glufosinate.

Although *barnase* expression is driven by a tissue-specific promoter, the applicant designed a system to prophylactically express *barstar* under a constitutive promoter in order to counteract the expression of *barnase* outside the anther. Thus, the GMO Panel assumed that the unintended *barnase* expression cannot be excluded and could potentially lead to the formation of a Barnase–Barstar complex elsewhere in the MS11 plant. Additional ELISA-derived data submitted by the applicant using purified Barnase and Barstar proteins indicated that when Barnase and Barstar are complexed and under the conditions of the performed ELISA, (i) 35.5% of the complexed Barnase protein amount can be measured and (ii) the Barstar protein is undetectable. Based on the above elements, the applicant provided estimated values of the highest possible levels of Barnase and Barstar in the different plant tissues taking into account both the complexed and the uncomplexed fractions of these proteins.^{9,10}

The mean values, standard deviations and ranges of protein expression levels in seeds of the PAT/bar, Barnase and Barstar proteins as determined by ELISA are summarised in Table 1. Based on the calculations performed by the applicant to estimate the highest possible levels for Barnase and Barstar in

⁸ Dossier: Part II – Section 1.2.2.3; 11/08/2017, 13/03/2018, 01/06/2018, 24/08/2018, 01/04/2019, 21/11/2019.

⁹ All ELISA measurements for Barnase were below the LLOQ (0.05 µg/g fresh weight) so in order to calculate the Barnase amount that could be complexed with Barstar, the LLOQ was considered as the maximum Barnase concentration and a correction factor of 35.5% (% Barnase that could be detected when in complex with Barstar) was applied to this value based on the ELISA performance.

¹⁰ The bound Barstar in the complex was calculated using the estimated bound Barnase, the molecular weights of Barnase and Barstar and the one-to-one molar ratio of Barnase/Barstar in the complex. To calculate the total Barstar levels, the unbound Barstar fraction was added to the complexed Barstar fraction. What was considered as the uncomplexed Barstar fraction was either any quantifiable Barstar measurement or the LLOQ (0.025 µg/g fresh weight) when the measurement was < LLOQ.

seeds, the total levels for these proteins were: Barnase = 0.14 µg/g fresh weight (0.159 µg/g dry weight based on the reported average 12% moisture)¹¹ and Barstar = 0.145 µg/g fresh weight (0.165 µg/g dry weight based on the reported average 12% moisture).¹² These estimated values together with the values of PAT/bar levels as determined by ELISA were used to estimate human and animal dietary exposure (see Section 3.4.5).

Table 1: Mean values, standard deviations and ranges of PAT/bar, Barnase and Barstar in seeds [µg/g fresh weight (fw) and µg/g dry weight (dw)] of oilseed rape MS11

	Glufosinate-ammonium treatment	
	Not treated	Treated
Seeds (maturity)		
PAT/bar^(a)	0.30 ± 0.17/0.34 ± 0.18 (Ranges FW: (0.06–0.56); DW: (0.06–0.59))	0.44 ± 0.18/0.49 ± 0.18 (Ranges FW: (0.27–0.77); DW: (0.31–0.84))
Barnase	< LLOQ ^(b)	< LLOQ
Barstar	< LLOQ	< LLOQ

(a): n = 9 and n = 15 for untreated and treated samples, respectively.

(b): LLOQ: Lower Limit of Quantification.

LLOQ for Barnase: 0.05 µg/g fw; LLOQ for Barstar: 0.025 µg/g fw.

3.2.5. Inheritance and stability of inserted DNA

Genetic stability of the oilseed rape MS11 insert was assessed by Southern analysis of genomic DNA from five generations and PCR-based and herbicide tolerance trait-based segregation analysis. For the Southern analysis, the restriction enzyme/probe combinations used were sufficient to conclude that all the plants tested retain the single copy of the insert and flanking regions, which were stably inherited in subsequent generations.

The results supported the presence of a single insertion, segregating in a Mendelian fashion.

3.2.6. Conclusions on molecular characterisation

The molecular characterisation data establish that oilseed rape MS11 contains a single insert consisting of one copy of the *barnase*, *barstar* and *pat/bar* expression cassettes. Bioinformatic analyses of the sequences encoding the newly expressed proteins and other ORFs present within the insert or spanning the junctions between the insert and genomic DNA did not indicate significant similarities to toxins and allergens. The stability of the inserted DNA and of the introduced herbicide tolerance trait was confirmed over several generations. The levels of the Barnase, Barstar and PAT/bar proteins were obtained and reported adequately.

The information provided on the protein characterisation indicate that the plant- and *E.coli*-produced Barnase, Barstar and PAT/bar proteins are equivalent and the *E.coli*-produced proteins can be used in the safety studies.

3.3. Comparative analysis

As described in Section 1, oilseed rape MS11 is intended to be used only to produce hybrid seed in the frame of the dedicated breeding system and thus not be commercialised as a stand-alone product for food and feed uses, import and processing. A challenging aspect for the risk assessment of oilseed rape MS11 is that this is a plant that is not designed to be cultivated for food and feed production as such but always stacked with a RF line. The breeding system with oilseed rape MS11 is intended to produce: (1) fertile hybrid oilseed rape (i.e. MS11 × RF3) seed (scenario 1, see Section 1); and (2) new oilseed rape MS11 seed to maintain the oilseed rape MS11 line (scenario 2, see Section 1). Neither of these two production scenarios is in line with the requirements laid down in Regulation (EU) No 503/2013, which was specifically developed to reflect cultivation conditions for food and feed uses, as opposed to breeding purposes. The GMO Panel notes how the characteristics of the introduced traits of oilseed rape MS11 challenge the comparative analysis to the extent that it is not possible to produce the materials and collect the data for the comparative analysis without deviating from the requirements laid down in Regulation (EU) No 503/2013. For this reason, deviations from scenario 1 for the commercial production of MS11 × RF3 hybrid seeds and scenario 2 for the maintenance of MS11 seeds were made by the applicant in order to conduct the comparative analysis.

For the purpose of assessing the comparative analysis, the GMO Panel considered two additional hypothetical scenarios (3 and 4 below) for the cultivation of MS11 for food and feed uses. The scenarios are based on application EFSA-GMO-BE-2016-138 and tailored on the specific management conditions used in the field trials for the cultivation of MS11 in the absence (scenario 3) or presence (scenario 4) of glufosinate-ammonium containing herbicides. In the two scenarios, oilseed rape MS11 is not crossed with oilseed rape RF3 or with the maintainer line: as a result, hybrid MS11 × RF3 seeds and MS11 seeds to maintain the line (the products of the original breeding system) are not produced, the only product being MS11 for food and feed uses. For this reason, and because they do not bring any agronomic advantage to the farmer, the two scenarios must be considered unrealistic.

- Scenario 3: a seed lot of oilseed rape MS11 (50:50 mixture of seeds containing/not containing the intended traits) is sown for cultivation. Glufosinate-ammonium is not applied. The MS11 plants will be pollinated by the male fertile plants not containing MS11 that are present within the seed lot at sowing. The harvest will be constituted by genetically homogeneous seeds except for event MS11 which will be present in 25% of the seeds. This is considered a theoretical scenario because, from an agronomic point of view, there is no advantage in sowing seeds containing an MS trait, where the seed set is always dependent on the availability of pollen from fertile plants.
- Scenario 4: a seed lot of oilseed rape MS11 (50:50 mixture of seeds containing/not containing the intended traits) is sown for cultivation. Glufosinate-ammonium is applied and 50% of the MS plants (those without the intended trait) is selectively destroyed. The remaining sterile, glufosinate-ammonium-tolerant MS11 plants will produce seeds only if pollinated by fertile oilseed rape plants that are present in the neighbouring fields. Because of the dynamics of pollen transfer for oilseed rape and of the requirement for synchronous flowering to occur, the extent of seed formation could be significantly reduced compared to scenarios 1, 2 and 3. In addition, the seeds produced will have a genetic background influenced by the genotype of the pollen that fertilised the MS11 plant and only 50% of them will be harbouring event MS11. This is considered a theoretical scenario because it would expose the farmer to a low/no yield scenario and is therefore agronomically unrealistic.

3.3.1. Overview of studies conducted for comparative analysis

Application EFSA-GMO-BE-2016-138 presents data on agronomic/phenotypic characteristics as well as on forage and seed composition of oilseed rape MS11 (see Table 2). The field trial studies, conducted in support of application EFSA-GMO-BE-2016-138, correspond to the cultivation scenarios 3 and 4 described above. In addition, the application contains data on seed characteristics of oilseed rape MS11.

Table 2: Main comparative analysis studies to characterise the GM oilseed rape MS11 provided in application EFSA-GMO-BE-2016-138

Study focus	Study details	Comparator	Non-GM reference varieties
Agronomic and phenotypic analysis	Field study, 6 sites ^(a) in Canada and 3 sites ^(b) in USA in 2014 and additional 4 sites ^(c) in Canada in 2016	N90-740	6 ^(d)
Compositional analysis	Field study, 6 sites ^(a) in Canada and 3 sites ^(b) in USA in 2014		

(a): The 2014 Canadian field trials were located in Wakaw, Saskatchewan; Gibbons, Alberta; MacGregor, Manitoba; Starbuck, Manitoba; Minto, Manitoba and Saskatoon, Saskatchewan.

(b): The 2014 US field trials were located in Northwood, North Dakota; Jerome, Idaho and Ephrata, Washington. A field trial established in Gardner, North Dakota and was removed from the study due to unfavourable weather conditions resulting in poor emergence.

(c): The 2016 Canadian field trials were located in St-Marc-sur-Richelieu Quebec; Bon Accord, Alberta; Hepburn Saskatchewan and Elm Creek Manitoba.

(d): The oilseed rape non-GM reference lines used in the field trials are: 46A65, AC Elect, AC Excel, Peace, Spectrum and Westar.

3.3.2. Experimental field trial design and statistical analysis

At each field trial site, the following materials were grown: oilseed rape MS11, the comparator N90-740 and three commercial non-GM oilseed rape reference varieties, all treated with conventional

herbicides management regimes; and oilseed rape MS11 exposed to the intended glufosinate-ammonium-containing herbicide, in addition to the conventional herbicides.

The GMO Panel makes the following remarks on the adequacy of the data for oilseed rape MS11:

- Data from F₁ plants, i.e. all agronomic-phenotypic endpoints except those harvest-related. The data for oilseed rape MS11 are considered adequate, as 50% of the oilseed rape MS11 plants contain the intended traits (however, early stand count is an exception because of double seed density, see Section 3.3.4.3).
- Data from F₂ seeds, i.e. all compositional endpoints and the harvest-related agronomic endpoints. Two cases must be considered, depending on herbicide treatment:
 - For plots with MS11 not treated with the intended herbicide (see hypothetical scenario 3), event MS11 will be present in 25% of the harvested seeds. From the context of a comparative assessment, it is considered that these data are adequate under the specific conditions of this application and thus, all the endpoints collected during the life cycle of the MS11 plants are adequate.
 - For plots with MS11 treated with the intended herbicide (see hypothetical scenario 4), event MS11 will be present in 50% of the harvested seeds. However, the genetic background of the seeds will be heterogeneous and location-dependent, as it will be influenced by the genotype of the pollen produced from nearby plots (oilseed rape N90-740 and the reference varieties present at the specific site). For this reason, harvest-related agronomic data and compositional data for MS11 treated with the intended herbicide are not considered adequate for the comparative analysis.

The agronomic, phenotypic and compositional data were analysed by the applicant as specified by the EFSA GMO Panel (2010b, 2011b). This includes, for each of the two treatments of oilseed rape MS11, the application of a difference test (between the GM oilseed rape and its comparator) and an equivalence test (between the GM oilseed rape and the set of non-GM-oilseed rape reference varieties).¹¹ The results of the equivalence test are categorised into four possible outcomes (I-IV, ranging from equivalence to non-equivalence).¹²

3.3.3. Suitability of selected test materials

3.3.3.1. Selection of the GM oilseed rape line and comparator

To obtain the GM oilseed rape MS11, the conventional oilseed rape N90-740 was used as recipient line for the transformation. Due to the nature of the MS trait, the obtained T₀ MS11 hemizygous line was backcrossed with the fertile non-GM oilseed rape line N90-740 (maintainer) to produce the T₁ generation. This process was repeated to produce the T₄ generation that was used for the comparative studies reported in Table 2. Oilseed rape N90-740 was not only used as the maintainer line but was also selected as the comparator being genetically as close as possible to the produced MS11 line. While oilseed rape N90-740 is fertile and this makes its life cycle different compared with MS11, the GMO Panel has evaluated the biological differences between oilseed rape N90-740 and MS11 and in light of the close genetic similarity considers oilseed rape N90-740 as an acceptable comparator.

The GM MS11 oilseed rape cannot be considered a representative line for commercial cultivation since, as a single event, it will not be cultivated by farmers (see Section 1). However, to conduct the comparative assessment, the selection of the MS11 line in the N90-740 genetic background is considered appropriate for the scope of these analyses. However, the GMO Panel notes that the MS11 seeds produced under scenario 4 are characterised by a heterogeneous genetic background (instead of N90-740), as influenced by the genotype of the pollen produced from nearby plots, and thus, they are not suitable for the comparative analysis (see Section 3.3.2).

¹¹ The purpose of the test of equivalence is to evaluate the estimated mean values for oilseed rape MS11 taking into account natural variability as defined by a set of commercial non-GM oilseed rape reference varieties with a history of safe use for consumption as food or feed.

¹² 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).

3.3.3.2. Selection of commercial non-GM oilseed rape reference varieties

To conduct the comparative field trials, six non-GM reference varieties (see Table 2) were selected. Three reference varieties were grown at each site of the field trials conducted in 2014 and 2016. The selected non-GM reference varieties are commercial oilseed rape lines present in national and international lists of varieties. The GMO Panel notes that non-GM sterile lines are not commercially available. It is therefore considered that the selected reference varieties were acceptable to support the comparative analysis, in line with the Regulation (EC) No 503/2013.

3.3.3.3. Seed production and quality

The seeds of GM oilseed rape MS11 and the conventional counterpart used in the field trials (see Table 2) were produced, harvested and stored under similar conditions. As described in the Introduction, under this breeding system, MS11 seed production leads to seed lots constituted by an equal mix of MS11 and non-GM-segregant seeds. The seed lots were verified for their purity via event-specific quantitative polymerase chain reaction analysis. The seeds were tested for their germination capacity at two different temperatures.¹³ The data obtained from seed germination studies were analysed with Fisher's Exact test. The results of these studies indicate that the seed germination of GM oilseed rape MS11 is not different than that of the conventional counterpart (N90-740). The GMO Panel considers that the starting seed used as test material in the agronomic, phenotypic and compositional studies was of suitable quality.

3.3.3.4. Conclusion on suitability

The GMO Panel is of the opinion that oilseed rape MS11, its conventional counterpart and the selected non-GM oilseed rape reference varieties are of adequate quality. The GMO Panel considers part of the selected materials acceptable considering the presence of the MS trait in the MS11 line for which the cultivation for food and feed uses only represents a theoretical scenario (see Introduction and above). However, MS11 seeds produced under scenario 4 are not considered appropriate for the comparative analysis.

3.3.4. Appropriateness of the receiving environments

MS11 is a line that does not represent an agronomically valuable material for food and feed cultivation and it is not expected to be grown by farmers. Therefore, the cultivation of MS11 line for food and feed production represents *per se* a lack of representativeness. In this specific context, the GMO Panel assessed the appropriateness of the selected field trials considering the theoretical cultivation scenarios 3 and 4 described in Section 3.3.

3.3.4.1. Selection of field trial sites

The selected field trial sites were located in commercial oilseed rape-growing regions of North America and Canada. Soil and climatic characteristics of the field trial sites are diverse,¹⁴ corresponding to optimal, near-optimal and suboptimal conditions for oilseed rape cultivation (Sys et al., 1993). The GMO Panel considers that the selected sites reflect commercial oilseed rape-growing regions.

3.3.4.2. Meteorological conditions

Maximum and minimum mean temperatures and sum of precipitations were provided on a monthly basis for sites conducted in 2014 and on a weekly basis for sites conducted in 2016. Some exceptional weather conditions were reported at two of the selected sites.¹⁵ However, due to the lack of major impacts on plant growth at these sites, the GMO Panel considers that the exceptional weather conditions did not invalidate the selection of the field trial sites for the comparative analyses.

¹³ Test conducted following the AOSA guideline for seeds testing under warm and cold temperature. Warm temperature regime: cyclical temperature of 16 and 8 h at $20 \pm 5^\circ\text{C}/30 \pm 5^\circ\text{C}$ for 7 days in the dark. Cold temperature regime: constant temperature at $10 \pm 5^\circ\text{C}$ for 7 days followed by cyclical temperature of 16 and 8 h at $20 \pm 5^\circ\text{C}/30 \pm 5^\circ\text{C}$ for 7 days in the dark.

¹⁴ Soil types of the field trials were sandy loam, loam, clay, silt loam, sandy clay loam and loamy sand; soil organic matter ranged from 1.7% to 8.5%; pH ranged from 5.3 to 8.4; historical mean temperatures and sum of precipitations during the growing season ranged, respectively, from 12.4°C to 19.2°C and from 57 to 354 mm.

¹⁵ Excessive rains occurred at St-Marc-sur-Richelieu Quebec from 12/8/2016 to 21/8/2016 and Elm Creek Manitoba in 2016 from 25/5/2016 to 31/5/2016.

3.3.4.3. Management practices

The field trials included plots containing oilseed rape MS11,¹⁶ plots with the conventional counterpart and plots with non-GM oilseed rape reference varieties. Considering the occurrence of the MS and the glufosinate-tolerant traits, the field trials were managed largely according to local agricultural practices, but some specific deviations were introduced. In addition, the field trials included plots containing oilseed rape MS11 managed following the same practices and exposed to the intended glufosinate-ammonium-containing herbicide. The plots containing the oilseed rape MS11 plants were sown using the MS11 seed lot that are constituted by a mix of 50% hemizygous plants (male sterile and glufosinate-ammonium-tolerant) and 50% of negative plants for MS11 (fertile and glufosinate-ammonium-susceptible). The plots containing GM plants exposed to the intended herbicide were planted at double seed density compared to the plots exposed to conventional herbicides. This management practice was applied to compensate the loss of the 50% negative MS11 plants that were present in the MS11 plots. This agronomic practice represents a deviation from standard management practices under farm cultivation. For this reason, the GMO Panel considered that the endpoint early stand count for MS11 exposed to the intended herbicide is not adequate for the comparative analysis. The glufosinate-ammonium containing herbicide was applied at the growth-stage BBCH12-14.

3.3.4.4. Conclusion on the representativeness

The GMO Panel concludes that under the specific theoretical cultivation scenarios considered, the geographical locations, soil characteristics, meteorological conditions and management practices of the field trials including planting, harvesting and application of plant protection products are appropriate for conducting the comparative assessment of oilseed rape MS11.

3.3.5. Conclusion on the appropriateness of the data for the comparative analysis

Under the specific theoretical cultivation scenarios considered, the GMO Panel is of the opinion that the generated data are only partially in line with the requirements of Regulation (EC) No 503/2013. As described in Section 3.3.2, the seeds produced from MS11 treated with intended herbicide (scenario 4) have a heterogeneous genetic background influenced by the genotype of the pollen produced from nearby plots. Hence, the GMO Panel considered that the compositional data and the harvest-related agronomic data generated for MS11 treated with the intended herbicide are not adequate.

3.3.6. Agronomic and phenotypic endpoints

Thirteen¹⁷ agronomic and phenotypic endpoints, including data on biotic and abiotic stressors and herbicide injury, were collected from all field trials (see Table 2).¹⁸

Six of the endpoints¹⁹ were measured as categorical data and not considered suitable for a parametric analysis. For those endpoints, differences between the GM oilseed rape and its conventional counterpart were investigated with a generalised Cochran–Mantel–Haenszel (CMH) test. Statistically significant differences were identified between oilseed rape MS11 and its conventional counterpart for seedling vigour and abiotic stress rating (stage BBCH 30-39).²⁰ For these endpoints, the average values fell within the range of the commercial reference varieties.

¹⁶ The plots containing the oilseed rape MS11 plants, were constituted using seeds derived from a MS11 (sterile) plant crossed with line N90-740 (maintainer), these seeds are constituted by a mix of 50% hemizygous plants (male sterile and glufosinate tolerant) and 50% of negative plants for MS11 (fertile and sensitive to glufosinate).

¹⁷ Early stand count, final stand count, days to flowering (50% plants flower), days to flowering – 10% remains, days to maturity, average plant height, seed yield, seedling vigour, lodging resistance, pod shattering, disease stress rating (assessed at four different times during the growing season), insect stress rating (at four different times) and abiotic stress rating (at four different times).

¹⁸ Three additional endpoints (fruit count, thousand seed weight and herbicide injury stressor) were measured only in four field trial sites and not evaluated further. Data for sterile plant count were also collected from 8 study sites, but only used to confirm the efficacy of the male sterile trait.

¹⁹ Categorical endpoints: seedling vigour, lodging resistance, pod shattering, disease stress rating (assessed at four different times during the growing season), insect stress rating (at four different times) and abiotic stress rating (at four different times).

²⁰ Average rating for seedling vigour: 7.5 (oilseed rape MS11 treated with the target herbicide) and 6.6 (conventional counterpart). Average abiotic stress rating (stage BBCH 30-39): 2.1 (oilseed rape MS11 treated with the target herbicide) and 1.6 (conventional counterpart).

The test of difference and the test of equivalence were applied to the remaining seven endpoints,²¹ with the following results:

- For oilseed rape MS11 treated with conventional herbicides, the test of difference identified no statistically significant differences with the conventional counterpart.
- For oilseed rape MS11 treated with the intended herbicides, the GMO Panel considered only the results for the adequate endpoints for the comparative analysis (i.e. excluding early stand counts and all the endpoints collected after harvest, see Sections 3.3.2 and 3.3.4.3). Statistically significant differences were identified for six of those endpoints,²² which all fell under equivalence category I or II.²³

As explained in Section 3.3.4.3, the endpoint early stand count for oilseed rape MS11 treated with the intended herbicide was considered not adequate for the comparative analysis because the GM plants were planted at double seed density compared to the other plots. After spraying with the intended herbicide, the plant density was reduced to levels comparable with the other plots. This is confirmed by the fact that the endpoint final stand count fell under equivalence category I. Therefore, the GMO Panel considered that this unusual management practice did not have an impact on the comparative assessment.

3.3.7. Compositional analysis

As described in Section 3.3, the seeds produced from MS11 treated with intended herbicide (scenario 4) have a heterogeneous genetic background influenced by the genotype of the pollen produced from nearby plots. Hence, the compositional data for MS11 treated with intended herbicide cannot be considered adequate for the comparative analysis. Without data for such material, the compositional data set is considered incomplete and was not taken into account in the assessment. The GMO Panel is therefore not in the position to complete the assessment of the compositional analysis.

3.3.8. Conclusion on the comparative assessment

Under the specific theoretical cultivation scenarios considered, the GMO Panel concludes that none of the differences identified in the agronomic and phenotypic characteristics tested between oilseed rape MS11 and its conventional counterpart needs further assessment.

Because of the lack of an appropriate data set, the GMO Panel is not in the position to conclude on the compositional analysis and can therefore not complete the comparative analysis.

3.4. Food/feed safety assessment

3.4.1. Effects of processing

Because the GMO Panel is not in the position to conclude on the compositional analysis and therefore cannot complete the assessment of the comparative analysis, it is also not in the position to assess the effect of processing of oilseed rape MS11.

3.4.2. Influence of temperature and pH on newly expressed proteins⁷

Effects of temperature and pH on the newly expressed proteins in this GM oilseed rape have been previously evaluated by the GMO Panel (EFSA, 2006, 2007; EFSA GMO Panel, 2012, 2016a,b, 2017b, c). Additional studies addressing heat stability of Barnase/barstar complex and the PAT/bar protein were provided by the applicant (Appendix A). The outcome of these studies is consistent with similar studies previously assessed by the GMO Panel.

²¹ Early stand count, final stand count, days to flowering (50% plants flower), days to flowering – 10% remains, days to maturity, average plant height and seed yield.

²² Final stand count, days to flowering (50% plants flower), days to flowering – 10% remains, days to maturity, average plant height.

²³ Results for early stand count (plants/plot) were as follows: mean level for the comparator: 141.98; mean level for the GM oilseed rape (treated with the intended herbicide): 263.74; equivalence limits: (87.00, 173.03).

3.4.3. Toxicology

3.4.3.1. Testing of the newly expressed proteins

The three proteins newly expressed in oilseed rape MS11 (Barnase, Barstar and PAT/bar) have been characterised in the context of this application (Section 3.2.3).

The PAT/bar protein was previously assessed by the GMO Panel in the context of other applications (e.g. EFSA, 2005, 2006, 2007; EFSA GMO Panel, 2013, 2016a,b) and no safety concerns for humans and animals were identified. Updated bioinformatics analyses reveal no similarities of the PAT/bar protein with known toxins. Additional studies addressing *in vitro* degradation of the PAT/bar protein were provided by the applicant (Appendix A). The outcome of these studies is consistent with previous studies assessed by the GMO Panel (EFSA, 2006, 2007; EFSA GMO Panel, 2016a,b, 2017b,c). The GMO Panel is not aware of any new information that would change previous conclusion on the safety of PAT/bar proteins.

The Barnase and Barstar proteins have been already evaluated by the GMO Panel in the context of previous applications (i.e. EFSA, 2005; EFSA GMO Panel, 2012, 2016b, 2017b,c) and not considered to pose risk for food and feed, due to the specificity of the molecular transgenic constructs (time/tissue-specific expression).

The GMO Panel noted that the *barstar* expression cassette in Ms11 differs from those previously assessed; being under a constitutive promoter to counteract the possible unintended Barnase expression in other plant tissues including those relevant for food and feed (Section 3.2.4).

A weight-of-evidence approach was followed in the context of the present application to exclude potential safety concerns for human and animal health of Barnase and Barstar proteins and their complex, taking into account the overall information presented in the dossier.

Updated bioinformatics analyses reveal no similarities of the Barnase and Barstar proteins known to be toxins. Additional studies addressing *in vitro* degradation of the Barnase and Barstar proteins were provided by the applicant (Appendix A). No indications of safety concern were identified by the GMO Panel. The outcome of these studies is consistent with previous studies assessed by the EFSA GMO Panel (EFSA GMO Panel, 2017b,c).

The available toxicological studies provided (acute oral gavage up to the dose of 2000 mg/kg body weight to male and/or female mice administered microbially produced Barnase and Barstar proteins and their combination; and 28 days by oral gavage⁷, alone or as a complex, respectively, up to the highest doses of 9.5, 10 and 20 mg/kg body weight (bw) to male and female CD-1 mice did not reveal adverse effects (Appendix A).

The GMO Panel does not identify concerns regarding the potential toxicity of the Barnase, Barstar as expressed in this oilseed rape MS11.

Based on scientific knowledge, no synergistic or antagonistic interactions raising food/feed safety concerns exist between the PAT/bar and the Barnase or Barstar proteins and their complex.

3.4.3.2. Testing of new constituents other than the newly expressed proteins

Since the GMO Panel is not in the position to conclude on the compositional analysis and therefore cannot complete the assessment of the comparative analysis, it is also not in the position to complete the assessment of new constituents other than the newly expressed proteins.

3.4.3.3. Information on altered levels of food/feed constituents

Since the GMO Panel is not in the position to conclude on the comparative analysis and therefore cannot complete the assessment of the compositional analysis, it is also not in the position to complete the assessment of altered levels of constituents.

3.4.3.4. Testing of the whole genetically modified food/feed

In accordance with Regulation (EU) No 503/2013, the applicant provided a 90-day oral repeated-dose toxicity study on whole food/feed from oilseed rape MS11 in rats.

Since the GMO Panel is not in the position to conclude on comparative analysis, is not possible to establish whether the 90-day study on Ms11 should be conducted according to a hypothesis-driven design or not (EFSA, 2014); therefore, the GMO Panel did not assess the 90-day study provided in the context of this application.

3.4.4. Allergenicity

The strategies to assess the potential risk of allergenicity focus on the source of the recombinant protein, on the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons and on whether the transformation may have altered the allergenic properties of the modified plant.

3.4.4.1. Assessment of allergenicity of the newly expressed proteins²⁴

A weight-of-evidence approach was followed, taking into account all the information obtained on the newly expressed protein, as no single piece of information or experimental method yield sufficient evidence to predict allergenicity (Codex Alimentarius 2009; EFSA GMO Panel, 2011a; Regulation (EU) No 503/2013).

The *pat/bar* gene originates from *S. hygroscopicus*, and the *barnase* and *barstar* genes originate from *B. amyloliquefaciens*, none of which are considered allergenic sources.

Updated bioinformatic analyses of the amino acid sequences of the Barnase, Barstar and PAT/bar proteins, using the criterion of 35% identity in a sliding window of 80 amino acids, revealed no significant similarities to known allergens. In addition, the applicant performed analyses searching for matches of eight contiguous identical amino acid sequences between these newly expressed protein sequences and known allergens.²⁵ The studies on resistance of the Barnase, Barstar and PAT/bar proteins to degradation by pepsin have been described in Section 3.4.3.1.

The GMO Panel has previously evaluated the safety of the Barnase, Barstar and PAT/bar proteins and no concerns on allergenicity were identified (EFSA 2006, 2007; EFSA GMO Panel 2012, 2016a,b, 2017b,c). As described above, it is also noted that the expression levels of Barnase and Barstar proteins were below the LLOQ in oilseed rape MS11 tissues relevant for food and feed (i.e. seeds). Nevertheless, expression levels of Barnase and Barstar above LLOQ cannot be completely excluded, and a theoretical estimation of the maximum possible levels of Barnase and Barstar proteins was considered (see Sections 3.2.4 and 3.4.5).

In addition, no information available on the structure or function of the newly expressed Barnase, Barstar and/or PAT/bar proteins would suggest an adjuvant effect of these proteins in oilseed rape MS11, resulting in or increasing an eventual immunoglobulin E (IgE) response to a bystander protein.

In the context of this application, the GMO Panel considers that there are no indications that the newly expressed Barnase, Barstar and/or PAT/bar proteins in oilseed rape MS11 may be allergenic.

3.4.4.2. Assessment of allergenicity of the whole GM plant

Since the GMO Panel is not in the position to conclude on the compositional analysis and therefore cannot complete the assessment of the comparative analysis, it is also not in the position to complete the assessment of allergenicity of the whole GM plant.

3.4.5. Dietary exposure assessment of new constituents

In line with Regulation (EU) No 503/2013, dietary exposure to PAT/bar, Barnase and Barstar proteins newly expressed in oilseed rape MS11 was estimated. Since oilseed rape MS11 is intended to be used only for the production of hybrid seed and therefore is not expected to enter the food and feed chain in the EU, except in the case of accidental presence in products coming from non-EU countries, the dietary exposure scenarios shown below are considered to be overly conservative.

Dietary exposure was estimated based on protein expression levels reported for oilseed rape MS11 treated with the intended herbicide, the current available consumption data and feed practices, the foods and feeds currently available in the market and the described processing conditions. All seed samples analysed for Barnase and Barstar were below LLOQ. As a worst-case scenario, these LLOQs as well as the information reported on the complexed and the uncomplexed fractions of these proteins (see Section 3.2.4) were used when deriving the concentration values used for the exposure estimations.

3.4.5.1. Human dietary exposure

Different food processed commodities can be produced from oilseed rape: protein-based products (flours, concentrates, isolates), pollen supplements and oil.

²⁴ Dossier: Part II – Section 1.5.1, 1.5.3 and additional information 24/1/2020.

²⁵ Additional information 24/1/2020 (included in the ORF analysis).

Exposure to PAT/bar, Barnase and Barstar proteins from the consumption of refined oil is expected to be negligible since the processing of rape seeds to oil of food grade quality leads to the removal of proteins. Likewise, oilseed rape MS11 is a male sterile rapeseed not producing any pollen; therefore, pollen supplements would not contain any pollen from this crop. Regarding protein-based products from oilseed rape, they might be used as protein/amino acids supplements and/or in a wide variety of food applications (meat imitates, baked goods, ready-to-eat drinks, etc.). Taking into account the intended use of oilseed rape MS11 (production of hybrid seed), the production of protein/amino acids supplements from these seeds is highly unrealistic and, therefore, they were not considered in the dietary exposure estimations. On the other hand, although being an overly conservative scenario, the GMO Panel considers feasible the use of flours/concentrates containing oilseed rape MS11 to produce meat imitates. Making use of the available consumption data on meat imitates in the EFSA Comprehensive European Food Consumption Database (reported as 'Textured soy protein'),²⁶ the GMO Panel requested to estimate chronic and acute dietary exposure to PAT/bar, Barnase and Barstar proteins assuming that all meat imitates are made of oilseed rape MS11.

Initial concentrations for Barnase and Barstar in seeds were derived as explained in Section 3.2.4, 0.14 µg/g for Barnase and 0.145 µg/g for Barstar, both in fresh weight. For PAT/bar, the average concentration in seeds was 0.49 µg/g fw (Table 1). No losses in the newly expressed proteins during processing were considered; a factor (0.8) was applied on the average protein concentrations in the seeds before they were used to estimate dietary exposure through the consumption of meat imitates.

The highest chronic dietary exposure in high consumers of meat imitates was estimated in the age class 'Other children', with estimates of 0.91, 0.29 and 0.30 mg/kg bw day for PAT/bar, Barnase and Barstar proteins, respectively. For the acute dietary exposure, high consumers of meat imitates would be exposed to 1.2, 0.38 and 0.40 mg/kg bw day of PAT/bar, Barnase and Barstar proteins, respectively, in 'Other children'.

3.4.5.2. Animal dietary exposure²⁷

Dietary exposure to PAT/bar, Barnase and Barstar proteins was provided by the applicant based on the consumption of canola meal and rape forage across different animal species. Estimations took into account protein levels in seeds and whole plant, as derived from replicated field trial sites in the 2014 Canada and US growing season.

Initial concentrations provided by the applicant for Barnase and Barstar proteins in seeds were revised, considering both the complexed and the uncomplexed fractions of these proteins, as explained in Section 3.2.4. The new data provided, together with those reported for the PAT/bar protein in Table 1, were used by the GMO Panel to estimate dietary exposure across different animal species (e.g. cattle, sheep, swine and poultry). The assessment was focused on the consumption of canola meal, and estimates for animal body weight, daily feed intake and inclusion rates of canola meal, as recommended by OECD for the EU animal population (OECD, 2013). To estimate the mean PAT/bar, Barnase and Barstar protein levels in canola meal, a factor of 1.6-fold was applied based on the protein content in this feed material relative to canola seed (OECD, 2011), assuming that no losses of newly expressed protein occur during processing. Estimated dietary exposure in livestock is reported in Table 3.

Table 3: Dietary exposure to PAT/bar, Barnase and Barstar proteins (µg/kg bw per day) in livestock, based on the consumption of canola meal

	Dietary exposure (µg/kg bw per day)		
	PAT/bar	Barnase	Barstar
Dairy cattle	3.01	0.97	1.01
Breeding swine	3.61	1.17	1.21
Finishing swine	4.70	1.52	1.58
Broiler	9.96	3.22	3.35
Layer	5.36	1.73	1.80
Turkey	11.2	3.62	3.77

²⁶ Available online: <https://www.efsa.europa.eu/en/applications/gmo/tools> [Accessed: January 13, 2020]

²⁷ Dossier: Part II – Section 1.6.2 and additional information: 26/11/2019; 4/3/2020.

3.4.6. Nutritional assessment of endogenous constituents

Because the GMO Panel is not in the position to conclude on the compositional analysis and therefore cannot complete the assessment of the comparative analysis, it is also not in the position to complete the nutritional assessment of GM food/feed.

3.4.7. Post-market monitoring of GM food/feed

Because the GMO Panel is not in the position to conclude on the compositional analysis and therefore cannot complete the assessment of the comparative analysis, and consequently on the food and feed safety, it is also not in the position to comment on post-market monitoring of the GM food/feed.

3.4.8. Conclusion on the food/feed safety assessment

The GMO Panel does not identify safety concerns regarding the potential toxicity and allergenicity of the Barnase, Barstar and PAT/bar proteins as expressed in this oilseed rape MS11.

Due to the incompleteness of the comparative analysis, the GMO Panel is not in the position to complete the toxicological, allergenicity and nutritional assessment of oilseed rape MS11.

3.5. Environmental risk assessment and monitoring plan²⁸

3.5.1. Environmental risk assessment

Considering the scope of application EFSA-GMO-BE-2016-138, which excludes cultivation, the environmental risk assessment (ERA) of oilseed rape MS11 mainly takes into account: (1) the exposure of microorganisms to recombinant DNA in the gastrointestinal tract of animals fed GM material and of microorganisms present in environments exposed to faecal material of these animals (manure and faeces); and (2) the accidental release into the environment of viable oilseed rape MS11 seeds during transportation and/or processing (EFSA GMO Panel, 2010b).

3.5.1.1. Persistence and invasiveness of the GM plant

Oilseed rape (*Brassica napus*) is an annual 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). Oilseed rape seeds have the ability to survive in soils for more than 10 years (Hails et al., 1997; Lutman et al., 2004, 2005, 2008; Begg et al., 2006; Messéan et al., 2007; D'Hertefeldt et al., 2008; Gruber et al., 2008; Beckie and Warwick, 2010; Belter, 2016; Peltonen-Sainio et al., 2014) and demographic studies and surveys have shown the ability of oilseed rape (*B. napus*) seed to establish self-perpetuating populations outside agricultural areas, mainly in semi-natural and ruderal habitats in different countries (e.g. Devos et al., 2012; COGEM, 2013; Bauer-Panskus et al., 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; Pascher et al., 2017). Oilseed rape is generally regarded as an opportunistic species, which can take advantage of disturbed sites (e.g. mowed areas, semi-natural habitats) 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, Meffin et al., 2015) 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 (Pessel et al., 2001; Claessen et al., 2005a,b; Garnier et al., 2006; Elling et al., 2009; Pascher et al., 2010; Banks, 2014) and can have the characteristics of a weed or ruderal (Banks, 2014). 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 seed bank or shed by resident feral adult plants, or to redistribution of feral seed from one location to another (Pivard et al., 2008a,b; Banks, 2014; Bailleul et al., 2016). Banks (2014) showed that the substantial

²⁸ Dossier: Part II – Sections 5 and 6.

increase in small and large (100 to 1,000 plants) feral populations occurred throughout the studied area during study years in Scotland.

It is expected that the intended traits of oilseed rape MS11 will provide a selective disadvantage to oilseed rape plants, even when they are exposed to glufosinate-ammonium-containing herbicides. Oilseed rape MS11 expresses the *barnase* gene, which results in lack of viable pollen and thus male sterility. Consequently, oilseed rape MS11 plants cannot transmit genes through pollen, and can only set seed if pollinated by another oilseed rape plant, which substantially reduces the fitness of oilseed rape MS11 plants.

In conclusion, the GMO Panel considers that oilseed rape MS11 will have reduced ability to survive and establish feral populations under European environmental conditions in case of accidental release into the environment of viable oilseed rape MS11 seeds compared to conventional oilseed rape varieties.

3.5.1.2. 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 cross-pollination from feral plants originating from spilled seeds.

3.5.1.2.1. Plant-to-microorganism gene transfer

Genomic DNA can be a component of food and feed products derived from oilseed rape. It is well documented that such DNA becomes substantially degraded during processing and digestion in the human or animal gastrointestinal tract. However, bacteria in the digestive tract of humans and animals, and in other environments, may be exposed to fragments of DNA, including the recombinant fraction of such DNA.

Current scientific knowledge of recombination processes in bacteria suggests that horizontal transfer of non-mobile, chromosomally located DNA fragments between unrelated organisms (such as from plants to bacteria) is not likely to occur at detectable frequencies under natural conditions (for further details, see EFSA, 2009).

The only mechanism known to facilitate horizontal transfer of non-mobile, chromosomal DNA fragments to bacterial genomes is homologous recombination. This requires the presence of at least two stretches of DNA sequences that are similar in the recombining DNA molecules. In the case of sequence identity with the transgene itself, recombination would result in gene replacement. In the case of identity with two or more regions flanking recombinant DNA, recombination could result in the insertion of additional DNA sequences in bacteria and thus confer the potential for new properties.

In addition to homology-based recombination processes, at a lower transformation rate, the non-homologous end joining and microhomology-mediated end joining are theoretically possible (EFSA, 2009; Hülter and Wackernagel, 2008). Independently of the transfer mechanism, the GMO Panel did not identify a selective advantage that a theoretical HGT would provide to bacterial recipients in the environment.

The updated bioinformatic analysis of event MS11 revealed three pairs of DNA sequences with sufficient length and sequence identity to bacterial DNA (EFSA, 2017b). These are the two 3' g7 terminators from the octopine Ti plasmid of *A. tumefaciens*; the 3' *nos* terminator and the *P-nos* promoter of the nopaline Ti plasmid of *A. tumefaciens*; and *barstar* and *barnase* of the *B. amyloliquefaciens*. The *barstar* and *barnase* genes show sequence identity with DNA from *B. amyloliquefaciens*. Homologous recombination would only replace natural variants of such genes, and thus not conferring any new trait to bacterial recipients. Given the nature of the transferred DNA, a facilitated double homologous recombination would not provide a selective advantage to potential bacterial recipients. The GMO Panel identifies no safety concern linked to an unlikely but theoretically possible horizontal gene transfer.

In summary, there is no indication for an increased likelihood of horizontal transfer of DNA from oilseed rape MS11 to bacteria. Given the nature of the recombinant DNA, the GMO Panel identified no safety concern linked to an unlikely but theoretically possible HGT.

3.5.1.2.2. Plant-to-plant gene transfer

For plant-to-plant gene transfer to occur, imported GM oilseed rape seeds need to germinate and develop into plants in areas containing sympatric wild relatives and/or cultivated oilseed rape with synchronous flowering and environmental conditions favouring cross-pollination.

Oilseed rape is an open pollinating crop plant capable of cross-pollinating with other *Brassica* crops (Eastham and Sweet, 2002). It can also spontaneously hybridise with sexually compatible feral and wild relatives. Several hybrids between oilseed rape and wild relatives have been reported in the scientific literature. Evidence suggests that transgenes could readily introgress into *B. rapa*, *B. juncea* and *B. oleracea*, and is expected to be rare with *B. nigra*, *Hirschfeldia incana*, *Raphanus raphanistrum* and *Sinapis arvensis* (reviewed by FitzJohn et al., 2007; Devos et al., 2009; Liu et al., 2013; Ellstrand et al., 1999, 2013; Tang et al., 2018). Under field conditions, transgene introgression has only been confirmed for *B. rapa* (Hansen et al., 2001, 2003; Warwick et al., 2003; Jørgensen et al., 2004; Norris et al., 2004; Jørgensen, 2007; Warwick et al., 2008). 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 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; Yoshimura et al., 2006; Aono et al., 2006, 2011; Elling et al., 2009; Katsuta et al., 2015; Luijten et al., 2015).

The likelihood of gene flow from feral oilseed rape MS11 plants to sexually compatible feral plants and wild relatives will be substantially reduced compared to other feral oilseed rape plants, because oilseed rape MS11 plants are male sterility. Consequently, gene flow will be limited to seed dispersal, as feral oilseed rape MS11 plants can set seed if pollinated by other feral oilseed rape plants (see Section 3.5.1.1).

In conclusion, the GMO Panel considers that the likelihood of environmental effects because of the spread of genes from oilseed MS11 rape in Europe will be lower than that of conventional oilseed rape varieties.

3.5.1.3. Interactions of the GM plant with target organisms

Taking the scope of application EFSA-GMO-BE-2016-138 (no cultivation) and thus the absence of target organisms into account, potential interactions of feral oilseed rape MS11 plants arising from seed import spills with target organisms are not considered a relevant issue.

3.5.1.4. Interactions of the GM plant with non-target organisms

Given that environmental exposure of non-target organisms to spilled GM seeds or feral GM oilseed rape plants arising from spilled oilseed rape MS11 seeds is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM oilseed rape, potential interactions of the oilseed rape MS11 with non-target organisms are not considered by the GMO Panel to raise any environmental safety concern.

3.5.1.5. Interactions with the abiotic environment and biogeochemical cycles

Given that environmental exposure to spilled seeds or feral oilseed rape MS11 plants arising from seed import spills is limited, and because most proteins are degraded before entering the environment through faecal material of animals fed GM oilseed rape, potential interactions with the abiotic environment and biogeochemical cycles are not considered by the GMO Panel to raise any environmental safety concern.

3.5.2. Post-market environmental monitoring

The objectives of a PMEM plan, according to Annex VII of Directive 2001/18/EC, are to: (1) 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) 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 rationale of the PMEM plan provided by the applicant (EFSA GMO Panel, 2011b).

As the ERA does not identify potential adverse environmental effects from the oilseed rape MS11, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for oilseed rape MS11 includes: (1) the description of an approach involving operators (federations involved in 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 established by EuropaBio for the collection of information recorded by the various operators; and (3) the review of relevant 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 and a final report at the end of the authorisation period.

The GMO Panel considers that the PMEM plan proposed by the applicant is in line with the scope of the application. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

In the context of PMEM, the applicant could further improve future literature searches according to the GMO Panel recommendations given in Section 3.1.

3.5.3. Conclusions on the environmental risk assessment and monitoring plan

The GMO Panel concludes that oilseed rape MS11 will have reduced ability to persist under European environmental conditions compared to conventional oilseed rape varieties. Considering the scope of the application EFSA-GMO-BE-2016-138, interactions of feral oilseed rape MS11 plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from oilseed rape MS11 to bacteria does not indicate a safety concern. Therefore, considering the introduced traits, the outcome of the agronomic and phenotypic analysis and the routes and levels of exposure, the GMO Panel concludes that oilseed rape MS11 would not raise safety concerns in the event of accidental release of viable GM oilseed rape seeds into the environment.

The GMO Panel considers that the PMEM plan proposed by the applicant is in line with the scope of the application and agrees with the reporting intervals of the PMEM plan.

4. Conclusions

The GMO Panel was asked to carry out a scientific assessment of oilseed rape MS11, subject of the application EFSA-GMO-BE-2016-138. In line with the scope of this application which is for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003, the GMO Panel concludes that:

- The molecular characterisation data establish that oilseed rape MS11 contains a single insert consisting of one copy of the *barnase*, *barstar* and *pat/bar* expression cassettes. Bioinformatic analyses of the sequences encoding the newly expressed proteins and other ORFs present within the insert or spanning the junctions between the insert and genomic DNA do not indicate significant similarities to toxins and allergens. The stability of the inserted DNA and introduced herbicide trait was confirmed over several generations. The levels of the Barnase, Barstar and PAT/bar proteins were obtained and reported adequately. The information provided on the protein characterisation indicate that the plant- and *E.coli*-produced Barnase, Barstar and PAT/bar proteins are equivalent and the *E.coli*-produced proteins can be used in the safety studies.
- The characteristics of the introduced traits of oilseed rape MS11 challenge the comparative analysis to the extent that it is not possible to produce the materials and collect the data for the comparative analysis without deviating from the requirements laid down in Regulation (EU) No 503/2013. None of the differences between oilseed rape MS11 and its conventional counterpart identified in the agronomic and phenotypic characteristics tested under the specific theoretical cultivation scenarios needs further assessment. Because of the heterogeneous genetic background of the seeds produced from oilseed rape MS11 treated with the intended herbicide, the compositional data cannot be considered adequate for the comparative analysis. Hence, no conclusions can be drawn for the compositional analysis.
- No safety concerns are identified regarding the toxicity and allergenicity of the Barnase, Barstar and PAT/bar proteins, as expressed in oilseed rape MS11. Owing to the incompleteness of the compositional analysis, the toxicological, allergenicity and nutritional assessment of oilseed rape MS11 cannot be completed.
- Oilseed rape MS11 would not raise safety concerns in the event of accidental release of viable GM oilseed rape seeds into the environment.
- The post-market environmental monitoring (PMEM) plan proposed by the applicant is in line with the scope of the application and agrees with its reporting intervals.
- Based on the relevant publications identified through the literature searches, no safety issues pertaining to the uses of oilseed rape MS11 were identified. In the context of PMEM, the

applicant could further improve future literature searches according to the GMO Panel recommendations. In addition, the information in additional unpublished studies (Appendix A) does not raise any concern for human and animal health and the environment, regarding oilseed rape MS11.

- In conclusion, in the absence of an appropriate comparative assessment and considering the scope of application EFSA-GMO-BE-2016-138 as defined by the applicant (food and feed uses, import and processing), the GMO Panel is not in a position to complete its food/feed assessment of oilseed rape MS11. However, oilseed rape MS11 is unlikely to have any adverse effect on the environment in the context of the scope of the application.

The GMO Panel notes that oilseed rape MS11 is designed to be used only for the production of hybrid seed in the frame of a dedicated breeding system and thus is not expected to be commercialised as a stand-alone product for food/feed uses. Therefore, seeds harvested from oilseed rape MS11 are not expected to enter the food/feed chain except in the case of accidental presence in products coming from non-EU countries. In this context, the GMO Panel therefore notes the following:

- The conclusions on the molecular characterisation data remain unchanged;
- With regard to food and feed safety, no concerns have been identified regarding the toxicity and allergenicity of the Barnase, Barstar and PAT/bar proteins, as expressed in oilseed rape MS11. Human and animal exposure to food and feed containing, consisting of or derived from oilseed rape MS11 (single event) is only expected to occur through the accidental entry of oilseed rape MS11 seeds into the food and feed chain, leading to a scale of exposure substantially reduced compared to a stand-alone product. In addition, no variations in the level of compound(s) of this GM plant are expected based on the intended trait. Taking the above into account, it is considered that in case of accidental exposure, oilseed rape MS11 would not pose risk to humans and animals;
- The conclusions on ERA and PMEM remain unchanged, furthermore, the scale of environmental exposure through the accidental release of viable oilseed rape MS11 seeds during transportation and/or processing will be substantially reduced compared to a stand-alone product.

5. Documentation as provided to EFSA

- Letter from the Competent Authority of Belgium received on 16 December 2016 concerning a request for authorization of the placing on the market of Brassica napus MS11 submitted in accordance with Regulation (EC) No 1829/2003 by Bayer CropScience.
- Application EFSA-GMO-BE-2016-138 validated by EFSA, 08 March 2017.
- Request for supplementary information to the applicant, 22 March 2017.
- Receipt of spontaneous information from the applicant, 05 May 2017.
- Request for supplementary information to the applicant, 12 May 2017.
- Receipt of supplementary information from the applicant, 31 May 2017.
- Request for supplementary information to the applicant, 21 June 2017.
- Request for supplementary information to the applicant, 22 June 2017.
- Request for supplementary information to the applicant, 07 July 2017.
- Receipt of supplementary information from the applicant, 10 July 2017.
- Receipt of supplementary information from the applicant, 11 August 2017.
- Receipt of supplementary information from the applicant, 10 July 2017.
- Receipt of spontaneous information from the applicant, 26 September 2017.
- Request for supplementary information to the applicant, 26 September 2017.
- Receipt of supplementary information from the applicant, 04 October 2017.
- Request for supplementary information to the applicant, 13 November 2017.
- Receipt of spontaneous information from the applicant, 17 November 2017.
- Request for supplementary information to the applicant, 21 December 2017.
- Receipt of supplementary information from the applicant, 19 January 2018.
- Receipt of supplementary information from the applicant, 13 March 2018.
- Request for supplementary information to the applicant, 21 March 2018.
- Receipt of supplementary information from the applicant, 27 April 2018.
- Request for supplementary information to the applicant, 04 May 2018.
- Request for supplementary information to the applicant, 17 May 2018.

- Receipt of supplementary information from the applicant, 01 June 2018.
- Request for supplementary information to the applicant, 25 June 2018.
- Request for supplementary information to the applicant, 27 June 2018.
- Receipt of supplementary information from the applicant, 24 August 2018.
- Request for supplementary information to the applicant, 18 September 2018.
- Receipt of supplementary information from the applicant, 19 November 2018.
- Request for supplementary information to the applicant, 18 December 2018.
- Receipt of supplementary information from the applicant, 18 January 2019.
- Request for supplementary information to the applicant, 07 March 2019.
- Receipt of supplementary information from the applicant, 01 April 2019.
- Request for supplementary information to the applicant, 16 April 2019.
- Receipt of supplementary information from the applicant, 14 June 2019.
- Receipt of spontaneous information from the applicant, 25 June 2019.
- Request for supplementary information to the applicant, 02 August 2019.
- Receipt of supplementary information from the applicant, 10 September 2019.
- Request for supplementary information to the applicant, 11 October 2019.
- Receipt of supplementary information from the applicant, 21 November 2019.
- Request for supplementary information to the applicant, 26 November 2019.
- Receipt of supplementary information from the applicant, 24 January 2020.
- Receipt of spontaneous information from the applicant, 04 March 2020.

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Abbreviations

bp	base pair
ELISA	enzyme-linked immunosorbent assay
ERA	environmental risk assessment
GM	genetically modified
GMO	genetically modified organism
HGT	horizontal gene transfer
HR	homologous recombination
IgE	immunoglobulin E
Nos	nopaline synthase
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PAT	phosphinothricin acetyl-transferase
PMEM	post-market environmental monitoring
RF	restores fertility

Appendix A – List of additional studies performed by or on behalf of the applicant with regard to the evaluation of the safety of oilseed rape MS11 for humans, animal or the environment

Study identification	Title
M-365250-01-1	Barstar protein - Acute toxicity by oral gavage in mice
M-429793-01-1	Barstar protein <i>in vitro</i> digestibility study in human simulated gastric fluid at pH 1.2
M-429800-02-1	Barstar protein - <i>In vitro</i> digestibility study in human simulated intestinal fluid - Report amendment no 1 of final report (Amended: 2016-05-19)
M-440532-01-1	The heat stability of microbially produced Barnase assessed by SDS-PAGE and western blot analyses
M-461494-01-1	Recombinant PAT/ <i>bar</i> protein: Acute toxicity by oral gavage in female mice
M-468940-01-1	Recombinant Barnase/Barstar complex protein: Acute toxicity by oral gavage in mice
M-474414-01-1	Recombinant Barnase protein - Acute toxicity by oral gavage in mice
M-475319-01-1	PAT/ <i>bar</i> protein - Acute toxicity by oral gavage in mice
M-475710-01-1	The effect of temperature on microbially produced Barnase assessed by ELISA
M-476903-01-1	Recombinant Barnase/Barstar complex protein: <i>In vitro</i> digestibility study in human simulated gastric fluid at pH 1.2
M-476904-01-1	Recombinant barnase/barstar complex protein: <i>In vitro</i> digestibility study in human simulated intestinal fluid
M-477906-01-1	The effect of temperature on microbially produced Barnase/Barstar protein complex assessed by ELISA
M-497799-02-1	Barnase protein - Acute toxicity study by oral gavage in mice
M-499084-01-1	Barstar protein Acute toxicity study by oral gavage: in mice
M-541215-02-1	Quantitative protein expression analysis of barnase, barstar and PAT/ <i>bar</i> proteins in whole plant and raceme matrices over three generations of MS11 (BCS-BNØ12-7) <i>Brassica napus</i> - Amended final report (Amended: 2016-04-25)
M-542702-01-1	MS11 x RF3, MS11, and RF3 <i>Brassica napus</i> - Protein expression analyses of field samples grown in Canada and the USA during 2014
M-549078-01-1	MS11 <i>B. napus</i> - Agronomic assessment of MS11 <i>B. napus</i> grown in Canada and the USA during 2014
M-549080-01-1	MS11 <i>B. napus</i> - Composition analysis of field samples grown in Canada and the USA during 2014
M-553788-01-1	MS11 <i>B. napus</i> - Summary of agronomic assessment of MS11 <i>B. napus</i> grown in Canada and the USA during 2014
M-553793-01-1	MS11 <i>B. napus</i> - Summary of composition analysis of field samples grown in Canada and the USA during 2014
M-553797-01-1	MS11 <i>B. napus</i> - Summary of protein expression analyses of field samples grown in Canada and the USA during 2014
M-556581-02-1	Channel catfish feeding study with MS11 canola - amended final report
M-557508-01-1	The effect of temperature on PAT/ <i>bar</i> as assessed by ELISA
M-557889-01-1	Broiler chicken feeding study with MS11 canola