Environmental risk assessment of import and processing of genetically modified oilseed rape MS11 x RF3 x MON 88302

COGEM advice CGM/260521-01

- The present application (EFSA/GMO/NL/2020/167) concerns the authorisation for the import and processing of genetically modified (GM) oilseed rape MS11 x RF3 x MON 88302, and its possible subcombinations, for use in food and feed;
- The stacked event MS11 x RF3 x MON 88302 was produced by conventional crossbreeding of the three GM parental oilseed rape lines;
- The GM oilseed rape expresses genes of a pollination control system, the *barnase* gene (MS11) which confers male sterility, and two copies of the *barstar* gene (MS11 and RF3) that restores male fertility and enhances transformation efficiency. The GM oilseed rape also expresses two copies of the *bar* gene (MS11 and RF3) and one copy of *cp4 epsps* (MON88302), which confer tolerance to glufosinate-ammonium- and glyphosate-containing herbicides, respectively.

- The molecular characterisation of MS11 x RF3 x MON 88302 meets COGEM's criteria;
- The phenotypic and agronomic characteristics of MS11 x RF3 x MON 88302 are comparable to conventional oilseed rape;
- The phenotypic and agronomic characterisation does not give any indication that MS11 x RF3 x MON 88302 is able to grow in other habitats than conventional oilseed rape under natural conditions, except in places where glyphosate- and glufosinate-ammonium-containing herbicides are used for weed control.

- Feral oilseed rape populations occur across the Netherlands, with a small number of plants (25 or less) per location, along distribution routes and handling areas as a result of spillage of oilseed rape seeds during transport and transshipment;
- Oilseed rape can hybridise with *Brassica rapa* which is a common plant along Dutch roadsides. To a lesser extent, it can also hybridise with *Brassica juncea* and *Brassica oleracea*;
- Stable incorporation (introgression) of genes from *B. napus* into wild *B. rapa* and feral *B. napus* populations may be possible.

- Accidental spillage during transshipment and transport of GM oilseed rape seeds may lead to the establishment of feral oilseed rape populations. This could lead to feral GM *B. rapa* plants with for instance herbicide tolerance;
- COGEM is of the opinion that the post-market environmental monitoring (PMEM) plan for GM oilseed rape should always include monitoring along transport routes (including roadsides and railway beddings), transshipment areas and introduction through bird feed mixtures. As these aspects are not included in the PMEM of MS11 x RF3 x MON 88302, COGEM cannot advise positively on the application, import, and processing of oilseed rape MS11 x RF3 x MON 88302 and its possible subcombinations for use in food and feed;
- COGEM abstains from giving advice on the potential risks of incidental consumption as a food/feed assessment is carried out by other organisations.

1. Introduction

The present application (EFSA/GMO/NL/2020/167), filed by BASF Agricultural Solutions Seed US LLC, concerns import and processing of genetically modified (GM) oilseed rape MS11 x RF3 x MON 88302, and all possible subcombinations, for use in food and feed. Oilseed rape MS11 x RF3 x MON 88302 was produced by conventional crossbreeding of the three GM parental lines. Two of the three GM parental lines (RF3, MON88302) have been authorised for import and processing for use in food and feed in the European Union.[1,2] MS11 is in the process of authorisation.[3,4]

GM oilseed rape MS11 x RF3 x MON 88302 is tolerant for glyphosate- and glufosinate-ammonium-containing herbicides, respectively by expressing the genes *cp4 epsps* (present in MON 88302) and *bar* (present in MS11 and RF3). It furthermore contains a pollination control system, namely the *barnase* gene (present in MS11) that confers male sterility, and two copies of the *barstar* gene (present in MS11 and RF3) which restores male fertility and enhances transformation efficiency.

2. Previous COGEM advice

COGEM issued a generic advice on aspects relevant to import and processing of GM oilseed rape in the Netherlands.[5] In addition, several advisory reports were issued on import and processing of GM oilseed rape lines with tolerance to various herbicides. COGEM was of the opinion that import and processing of these oilseed rape lines posed a negligible risk to the environment, but that the corresponding post-market environmental monitoring (PMEM) plan supplied by the applicant did not meet COGEM's requirements.[6,7,8,9,10,11,12,13] COGEM advised similarly on the import and processing of the parental lines MS11,[14,15] MON 88302[12,13,16], and RF3[6,17,18], and the import and processing of several stacked events containing parental lines of the stacked event under assessment in this advice, including MS8 x RF3,[17,18] MON 88302 x MS8 x RF3[8], MS11 x RF3[19] and MON 94100 x MON 88302 x RF3.[20] Because the application regarding import and processing of MS11 pertained a hypothetical situation that would not occur, COGEM had no objections to its PMEM as it was irrelevant.[14]

3. Environmental risk assessment

The objective of an environmental risk assessment (ERA) is to identify and evaluate potential adverse effects of the genetically modified organism (GMO), direct or indirect, immediate or delayed, on human health and the environment. This ERA involves the import of GM oilseed rape, any concerns relating to cultivation, management or harvesting practices are beyond the scope of this advice.

3.1 Characteristics of the oilseed rape

Oilseed rape (*Brassica napus*) is a member of the *Brassicaceae* family, which amongst others includes *Brassica rapa*, *Brassica juncea*, *Brassica oleracea* (cabbage), *Brassica nigra* (black mustard) and *Brassica carinata* (Ethiopian mustard). The allotetraploid *B. napus* is the result of natural hybridisation between *B. rapa* and *B. oleracea*.[5,21]

Brassica napus reproduces by self-pollination and cross-pollination. It produces large amounts of pollen, which are dispersed by both wind and insects. In fields, the average rate of cross-pollination is 30%. The seeds of *B. napus* are arranged in a single row in the fruit (a linear cylindrical silique), and are small, light and produced in large quantities.[5,22,23] Oilseed rape can cross-pollinate with its more common wild relative *B. rapa* and to a lesser extent with *B. juncea* and *B. oleracea*.[5,22]

3.2 Receiving environment

In the Netherlands, *B. napus* is grown as a crop and its seeds are imported for oil production. Feral *B. napus* populations grow on disturbed soil. The spillage of oilseed rape seeds during transport and trans-shipment has led to the establishment of feral populations, with a small number of plants (25 or less) per location, in disturbed environments along distribution routes and handling areas.[24]

Oilseed rape x *B. rapa* hybrid plants have been observed in the Netherlands.[25] Stable incorporation (introgression) of genes from *B. napus* into wild *B. rapa* has been reported in Canada, but has not been documented in the Netherlands.[26] In a survey performed in the Netherlands in 2019 to investigate the presence of GM oilseed rape along transport routes and locations where transhipment and processing of oilseed rape takes place, no GM oilseed rape was detected.[27,28] Recent investigation has shown that GM oilseed rape seeds can be present in bird feed mixtures.[29,30] This could pose a potential introduction route of GM oilseed rape into the Dutch environment.

Conclusion: Feral *B. napus* populations exist in the Netherlands. *Brassica napus* can hybridise with its wild relative *B. rapa*. Therefore, GM volunteers from spilled seeds can lead to dispersal of genes to wild *B. napus* and feral *B. rapa* populations. No GM oilseed rape has been reported in the Netherlands.

3.3 Description of the introduced genes and traits

Oilseed rape MS11 x RF3 x MON 88302 was created by conventional crossbreeding of the parental lines. A description of the inserted genetic elements is listed in the table below. The list is limited to information on the introduced genes, corresponding traits, and their regulatory elements (i.e. promoters and terminators). For a detailed description of the introduced genes, see references.

Table 1. *Description of the introduced genes and traits.*

Introduced genes	Encoded proteins	Regulatory elements	Traits
cp4 epsps (MON 88302)	CP4 5-enolpyruvylshiki-mate-3-phosphate synthase (EPSPS) originating from *Agrobacterium tumefaciens* strain CP4.[31]	Chimeric promoter FMV/Tsf1, derived from enhancer sequences from the figwort mosaic virus 35S RNA together with the promoter from the *Tsf1* gene of *Arabidopsis thaliana*; the 5'UTR leader sequence of *Tsf1 A. thaliana,* the CTP2 target sequence of the *shkG* gene, from *A. thaliana*; the 3' UTR of the rubisco subunit (rbcS2) E9 gene, from *Pisum sativum.*	Confers tolerance to glyphosate-containing herbicides.
bar (MS11 and RF3)	Phosphinothricin N-acetyl-transferase (PAT) enzyme from *Streptomyces hygroscopicus.*[32]	PssuAt promoter, from the rubisco small subunit gene in *A. thaliana*, and the transcript 7 gene 3'- untranslated region (Tg7 terminator) derived from *A. tumefaciens,* and a flanking genomic sequence from unknown origin.	Confers tolerance to glufosinate-ammonium-containing herbicides.

Introduced genes	Encoded proteins	Regulatory elements	Traits
barnase (MS11)	Encodes the Barnase ribonuclease protein, which is expressed in the anther tapetal cell layer during pollen development, originating from *Bacillus amyloliquefaciens*.[33]	The pollen specific promotor Pta29 from *Nicotiana tabacum* and the terminator of the *nopaline synthase* (*nos*) gene from *A. tumefaciens*, and a flanking genomic sequence from unknown origin.	Confers male sterility.
barstar (RF3)	Barstar ribonuclease-inhibitor protein, which is expressed in the anther tapetal cell layer during pollen development originating from *B. amyloliquefaciens.*[33]	The pollen specific promoter Pta29 from *N. tabacum* and the terminator of the *nos* gene from *A. tumefaciens*, and a flanking genomic sequence from unknown origin.	Restores male fertility.
barstar (MS11)	Barstar ribonuclease-inhibitor protein, originating from *B. amyloliquefaciens.*[33]	P-*nos* promotor of the nopaline Ti plasmid of *A. tumefaciens*, and and the transcript 7 gene 3'- untranslated region (Tg7 terminator) derived from *A. tumefaciens.*	Enhances transformation efficiency.

3.4 Molecular characterisation

Previously, COGEM evaluated the molecular characterisation of each parental line and considered these to be adequate.[14,10,6,17] The sequences of the inserts and flanking regions are identical to those in the corresponding parental lines.

The applicant updated the bioinformatic analyses of the inserts and the sequences spanning the insertion sites from stop-to-stop codon and the 5' and 3' flanking regions. The putative products of the open reading frames (ORFs) in all six reading frames spanning the inserts, and those within the inserts themselves, were evaluated for potential similarity with databases with known allergens, toxins and in the case of *cp4 epsps* biologically active proteins that may be harmful to human or animal health. No biologically relevant matches were found.

Conclusion: The molecular characterisation was conducted according to the criteria previously laid down by COGEM.[34] No indications were identified for potential environmental risks of the stacked event oilseed rape MS11 x RF3 x MON 88302.

3.4 Phenotypic and agronomic characteristics

The applicant evaluated the phenotypic and agronomic characteristics of oilseed rape MS11 x RF3 x MON 88302 in comparison to conventional counterparts. No differences were found in germination characteristics between MS11 x RF3 x MON 88302 and conventional oilseed rape. The applicant's evaluation of phenotypic and agronomic characteristics showed a significant difference between conventional counterpart and reference varieties regarding 'early stand count', however, mean values were within the range of the values observed for the reference varieties. Therefore, it is unlikely that

the differences increase the fitness of MS11 x RF3 x MON 88302 under natural conditions in comparison to conventional varieties.

In locations where glyphosate- or glufosinate-ammonium-containing herbicides are used for weed control, the introduced herbicide tolerance traits may give MS11 x RF3 x MON 88302 an advantage over other plants.

Conclusion: There are no indications that the introduced traits alter the fitness of oilseed rape MS11 x RF3 x MON 88302 under natural conditions.

4. Food/feed assessment

This application is submitted under Regulation (EC) 1829/2003, therefore a food/feed assessment is carried out by EFSA and national organisations involved in the assessment of food safety. In the Netherlands, a food and/or feed assessment for Regulation (EC) 1829/2003 applications is carried out by Wageningen Food Safety Research (WFSR). The outcome of the assessment by other organisations (EFSA, WFSR) was not known when this advice was completed.

5. Post-market environmental monitoring

The applicant supplied a general surveillance plan as part of the post-market environmental monitoring (PMEM). On multiple occasions, COGEM has expressed concerns regarding the PMEM plan of GM oilseed rape events. Feral oilseed rape populations can arise from GM oilseed rape seeds spilled during transshipment and transport.[35,36,37,38] Additionally, as GM oilseed rape seeds can potentially be introduced in the environment via bird feed mixtures, this introduction route should be considered in the PMEM plan.[29,30] Presence of feral GM oilseed rape could result in potential gene flow. As it cannot be excluded beforehand that such a newly generated stacked event may have an adverse effect, COGEM remains of the opinion that the monitoring plan for MS11 x RF3 x MON 88302 should include monitoring along transport routes and transshipment areas. Additionally, the monitoring plan should include producers and distributors of seed mixtures in the PMEM plan, and should offer a place where adverse effects, and unexpected or notable events are reported.

Conclusion: The current PMEM plan of MS11 x RF3 x MON 88302 does not include monitoring along transport routes and transshipment areas, or its introduction via bird feed mixtures. COGEM is of the opinion that the PMEM plan should be adapted before market authorisation is granted.

6. Overall conclusion

There are no indications that the expression of the introduced traits of GM oilseed rape MS11 x RF3 x MON 88302 poses an environmental risk under natural conditions. Import and processing of MS11 x RF3 x MON 88302 poses a negligible risk to human health and the Dutch environment. However, accidental spillage of seeds may lead the establishment of feral GM oilseed rape, including plants with stacked events. COGEM is of the opinion that the monitoring plan should include monitoring along transport routes and transshipment areas, and introduction via bird feed mixtures. Therefore, COGEM can only advise positively on the application for import and processing for use in food and feed of MS11 x RF3 x MON 88302 oilseed rape if the PMEM plan is elaborated. COGEM abstains from giving advice on the potential risks of incidental consumption since other organisations carry out a food/feed assessment.

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