Import and processing of genetically modified oilseed rape MS11

COGEM advice CGM/170519-01

- The present application (EFSA/GMO/BE/2016/138) concerns the authorisation for import and processing for use in feed and food and other products (containing or consisting) of genetically modified (GM) oilseed rape MS11;
- Oilseed rape MS11 is a male sterile line. It expresses the *barnase* gene which results in lack of viable pollen and male sterility. MS11 also contains the *barstar* gene which, according to the applicant, enhances the transformation frequency. And it expresses the *bar* gene, which confers tolerance to glufosinate-ammonium containing herbicides;

- 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 populations of *B. rapa* and *B. napus* may be possible;
- There are no indications that the introduced traits alter the fitness of oilseed rape MS11. Herbicide tolerance gives the crop advance in places where glufosinate-ammonium herbicides are used for weed control;

- In general, COGEM is of the opinion that the monitoring plan for feed, import and processing of GM oilseed rape events should include monitoring along transport routes (including roadsides and railway beddings) and transshipment areas;
- However, oilseed rape MS11 is a male sterile line that, according to the applicant, will not be commercialised as a stand-alone product and will only be used for the production of hybrid seed;
- Import of MS11 in the EU will not take place. Therefore, COGEM has no objections to current monitoring plan as it is irrelevant;

- COGEM is of the opinion that the hypothetical import and processing of oilseed rape MS11 poses a negligible risk to the environment in the Netherlands;
- COGEM abstains from giving advice on the potential risks of incidental consumption since a food/feed assessment is carried out by other organisations.

1. Introduction

The present application (EFSA/GMO/BE/2016/138), filed by Bayer CropScience LP, concerns import and processing of genetically modified (GM) oilseed rape MS11, for use in feed and food. MS11 was produced by *Agrobacterium tumefaciens* mediated transformation. The GM oilseed rape MS11 contains the *bar* gene conferring tolerance to glufosinate-ammonium containing herbicides by expressing the enzyme phosphinothricin N-acetyltransferase (PAT). It expresses the *barnase* gene which results in lack of viable pollen and male sterility. MS11 also contains the *barstar* gene which, according to the applicant, enhances the transformation frequency. According to the applicant, oilseed rape MS11 will not be commercialised as a stand-alone product, but will only be used for the production of hybrid seed.

2. Previous COGEM advices

COGEM has not yet advised on the import and processing of oilseed rape MS11. The COGEM has advised on the import, processing and cultivation of MS8xRF3 oilseed rape, which contains the same transgenic traits (*bar, barnase* and *barstar*) as MS11.[4] In 2013, COGEM issued a generic advice on aspects relevant for import and processing of GM oilseed rape in the Netherlands.[1]

In case of GM oilseed rape, COGEM is of the opinion that an elaborate post-market environmental monitoring (PMEM) plan is needed. In the view of COGEM, monitoring of oilseed rape transport routes (including roadsides and railway beddings) and transshipment areas is a prerequisite to grant an authorisation for import and processing of GM oilseed rape events. Multiple applications filed for import and processing of GM oilseed rape lacked such elaborate monitoring plans. Therefore, COGEM issued several opinions in which it has advised negatively on import and processing of GM oilseed rape events including the events MS8, RF3 and MS8xRF3.[2,3,4]

3. Environmental risk assessment

3.1 Aspects of the wild-type crop

Oilseed rape (*Brassica napus*) is a member of the *Brassicaceae* family, which also includes *Brassica rapa*, *Brassica juncea*, *Brassica oleracea* (cabbage), *Brassica nigra* (black mustard) and *Brassica carinata* (Ethiopian mustard). *B. napus* is a hybrid that originates from the interspecific hybridisation of *B. oleracea* and *B. rapa*.[1,5]

B. napus reproduces by self- and cross-pollination. It produces high 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* develop in a fruit, and are small, light and produced in large quantities. Oilseed rape seeds generally do not display dormancy when they leave the plant, but they can acquire so-called dark dormancy after burial. The seed bank of oilseed rape has quite a rapid turnover but a small portion of the seeds can remain viable for several years.[1,6,7]

In the Netherlands, *B. napus* is grown as a crop and its seeds are imported for oil production. Wild *B. napus* populations grow on disturbed soil. *B. napus* is able to form volunteers in distributed environments near roadsides, railways and handling areas. The spillage of oilseed rape seeds during

transport and transshipment has led to the establishment of feral populations, with a small number of plants (25 or less) per location, along distribution routes and handling areas.[8]
Oilseed rape can cross-pollinate with its more common wild relative *B. rapa* and to a lesser extent with *B. juncea* and *B. oleracea*. [1,6] Oilseed rape x *B. rapa* hybrid plants have been observed in the Netherlands.[9] Stable incorporation (introgression) of genes from *B. napus* into wild *B. rapa* has not been documented in the Netherlands, but has been reported in Canada.[10]

Conclusion: Wild *B. napus* populations exist in the Netherlands. *B. napus* can hybridise with its wild relative *B. rapa*. Therefore, GM volunteers from spilled seeds can lead to dispersal of genes to wild populations of *B. napus* and *B. rapa.*

3.2 Description of the introduced genes and traits

Oilseed rape MS11 was produced by means of *Agrobacterium tumefaciens* mediated transformation. The T-DNA contains a *barstar, barnase* and *bar* expression cassette. The plasmid backbone contains, amongst other things, an *aadA* antibiotic resistance gene, which is used as a selection marker.

Introduced genes	**Encoded proteins and expression pattern**	**Traits**
barnase	Encodes the Barnase ribonuclease protein, which is expressed in the anther tapetal cell layer during pollen development.[11]	Confers male sterility.
barstar	Encodes the tapetal-cell-specific Barstar ribonuclease-inhibitor protein.[12]	Inhibits the Barnase protein and enhances transformation frequency.
bar	Encodes the phosphinothricin N-acetyltransferase (PAT) enzyme.[13]	Tolerance to glufosinate-ammonium herbicides.
For a detailed description of the introduced genes and traits, see for example reference 11.		

The applicant states that MS11 is a male sterile line. COGEM notes that both the *barnase* and the *barstar* gene are present in MS11. Male sterility is caused by the presence of the Barnase protein in the tapetum cells, which results in lack of viable pollen. The applicant does not explain how male sterility is maintained in the presence of Barstar. COGEM considers this important information because Barstar is a known inhibitor of Barnase. Presumably, the low expression of the *barstar* gene is not sufficient to overcome male sterility, but counteracts leaky expression of the *barnase* gene in other cells than tapetum cells, thus enhancing transformation frequency.

For clarity purposes, COGEM is of the opinion that the applicant should describe in more detail how male sterility is maintained in this background. This information is, however, not relevant for the environmental risk assessment by COGEM.

3.3 Molecular characterisation

Southern blot analyses showed that oilseed rape MS11 contains one copy of the insert at a single integration locus and that there are no T-DNA fragments elsewhere in the MS11 genome. Southern blot analysis in combination with PCR analysis also demonstrated the absence of pTCO113 backbone sequences.

The applicant determined the sequence of the MS11 insert and adjacent flanking oilseed rape genomic DNA sequences. Sequence analysis of the T-DNA insert showed that MS11 contains intact *barstar, barnase* and *bar* expression cassettes. A comparison with the parental oilseed rape line revealed that a 40 bp deletion occurred in the oilseed rape genome as a result of T-DNA insertion.

Similarity searches indicated the presence of an endogenous gene in the 3' flanking sequence region of the MS11 insertion locus, but the coding sequence of this gene is not interrupted upon insertion of T-DNA sequences. The insertion of T-DNA sequences in the MS11 insertion locus is unlikely to interrupt or alter transcriptional or translational activity of known endogenous *B. napus* genes.

The applicant screened the junctions between the T-DNA insert and the flanking plant genomic DNA as well as the entire insert for potential newly created open reading frames (ORFs). The ORFs were defined as sequences between two stop codons with a coding capacity of minimal eight amino acids. The search identified 554 ORFs. These were evaluated for potential identity with known proteins. No biologically meaningful protein sequence similarities with toxic proteins were detected in these bioinformatics analyses.

The molecular characterisation was conducted according to the criteria previously laid down by COGEM.[14]

Conclusion: The molecular characterisation of oilseed rape MS11 is adequate and no indications for potential environmental risks were identified.

3.4 Phenotypic and agronomic characteristics

The applicant evaluated the phenotypic and agronomic characteristics of oilseed rape MS11 in comparison to its conventional counterpart and commercial reference oilseed rape varieties. The results of the phenotypic and agronomic evaluation do not give reason to assume that the introduced traits alter the fitness of oilseed rape MS11. Herbicide tolerance gives the crop advance in places where glufosinate-ammonium herbicides are used for weed control.

Therefore, COGEM is of the opinion that there are no indications that that this GM oilseed line poses an environmental risk.

Conclusion: There are no indications that the introduced traits alter the fitness of oilseed rape MS11 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 RIKILT. The outcome of the assessment by other organisations (EFSA, RIKILT) was not known when this advice was completed.

5. Post-market environmental monitoring (PMEM)

The applicant supplied a general surveillance plan as part of the PMEM. On several occasions, COGEM has expressed concerns with regard to the PMEM plan of GM oilseed rape events. COGEM is of the opinion that for GM oilseed rape events a PMEM plan is needed which includes monitoring along transport routes (including roadsides and railway beddings) and transshipment areas.[2,3,4]

MS11 is a male sterile line that does not produce pollen and is used for the production of hybrid seeds. Although an application for import and processing of MS11 was filed, it will not be commercialised as a single event. This peculiar situation results from the procedures followed by EFSA, i.e. that an application for import and processing of a stacked GM line can only be filed if the parental GM lines have been assessed.[15]

Import of MS11 GM oilseed rape event in the EU will not take place. Therefore, COGEM has no objections to the current monitoring plan as it is irrelevant. In the future, COGEM will continue to assess case-by-case whether the submitted general surveillance plan is adequate for that specific application. This also applies to future applications of stacked events with MS11.

6. Additional remark

EFSA has previously published a guidance document on the environmental risk assessment (ERA) of GM plants.[15] In the guidance document it is stated that for the risk assessment of stacked events, the risk assessment of the single events included in a stack is always a pre-requisite.

COGEM has previously noted that there are circumstances where assessing single events and sub-combinations of events included in a stack is not relevant.[16] COGEM recommended to only require an evaluation of single and different combinations of events, if these events will segregate in the field or in the environment, and if there is a scientific reason that there can be synergistic or antagonistic effects between the different events. In this way, needless administrative workload, costs and eventual delay in authorization processes is significantly precluded.[16]

COGEM points out that it is not relevant to assess the single event MS11, because it will never be commercialized as a stand-alone product. COGEM considers the request for authorization of MS11 for food and feed uses and import and processing as a striking example of following unnecessary procedures.

7. Overall conclusion

COGEM is of the opinion that the hypothetical import and processing of MS11 poses a negligible risk to the environment in the Netherlands. COGEM abstains from giving advice on the potential risks of incidental consumption since other organisations carry out a food/feed assessment.

References

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