Renewal of the authorisation for import and processing of genetically modified oilseed rape MS8, RF3 and MS8xRF3

COGEM advice CGM/170112-01

- The present application (EFSA/GMO/RX/004) concerns the renewal of the authorisation for import and processing for use in feed of genetically modified (GM) oilseed rape MS8, RF3 and MS8xRF3;
- Oilseed rape MS8xRF3 has previously been authorized for feed, import and processing in 2007 and food in 2013;
- In 2004, COGEM has advised positively on the cultivation, import and processing of oilseed rape MS8, RF3 and MS8xRF3;

- Oilseed rape MS8, RF3 and MS8xRF3 express genes of a pollination control system, i.e. the *barnase* gene (present in MS8) which confers male sterility and the *barstar* gene (present in RF3) which restores male fertility. They also express 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 of genes from *B. napus* into wild populations of *B. rapa* (introgression) may be possible;

- The molecular characterization and bioinformatics analyses of MS8, RF3 and MS8xRF3 have been updated and meet the criteria of COGEM;
- Oilseed rape MS8, RF3 and MS8xRF3 events themselves do not pose a risk to the environment in the Netherlands;
- There are no indications that the introduced traits alter the fitness of oilseed rape MS8, RF3 and MS8xRF3 under natural conditions, except in places where glufosinate-ammonium herbicides are used for weed control;
- In the long term, prolonged use of herbicides may lead to the establishment of feral herbicide tolerant GM *B. napus*, including plants with stacked events, or feral herbicide tolerant *B. rapa* harbouring GM traits;
- The monitoring plan does not meet the minimal requirements for feed, import and processing of MS8, RF3 and MS8xRF3 and should include monitoring along transport routes (including roadsides and railway beddings) and transshipment areas;
- Because of the insufficient monitoring plan COGEM cannot advise positively on the renewal application for feed, import and processing of MS8, RF3 and MS8xRF3 oil seed rape.

1. Introduction

The present application (EFSA/GMO/RX/004), filed by Bayer CropScience, concerns the renewal of the consent for feed, import and processing of the genetically modified (GM) oilseed rape events MS8, RF3 and MS8xRF3 and their use as any other conventional oilseed rape variety with the exception of cultivation and uses as or in food. The authorisation for food (2013/327/EU) is valid until 2023. GM oilseed rape MS8, RF3 and MS8xRF3 are tolerant for glufosinate-ammonium containing herbicides and express a pollination control system. MS8xRF3 has been authorised for feed, import and processing in 2007 (2007/232/EC). Since import and processing authorisations remain valid for a period of 10 years, the applicant filed an application for the renewal of the authorisation for feed, import and processing. The application contains amongst others monitoring reports, an updated molecular characterisation and an updated literature search.

2. Previous COGEM advices

COGEM has advised positively on the import, cultivation and processing of oilseed rape MS8xRF3 in 2004.[1] In 2009, COGEM concluded that an update of the molecular characterisation of RF3 was adequate and fulfilled the criteria of COGEM.[2] In 2013, COGEM issued an advisory report on aspects relevant for import and processing of GM oilseed rape in the Netherlands.[3]

In case of GM oilseed rape, COGEM is of the opinion that a more 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 all GM oilseed rape events. Multiple filed applications for import and processing of GM oilseed rape lacked more elaborate monitoring plans. Therefore COGEM issued several opinions in which it has advised negatively on import and processing of GM oilseed rape events.[4,5,6]

3. Environmental risk assessment

3.1 Aspects of the crop

Oilseed rape (*Brassica napus*) is a member of the *Brassicaceae* family (sya. *Cruciferae*), 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*.[6]

B. napus reproduces by self- and cross-pollination. It produces a huge amount of pollen which is 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.[6]

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.[7]

Oilseed rape can cross-pollinate with its more common wild relative *B. rapa* and to a lesser extent with *B. juncea* and *B. oleracea*.[6] Oilseed rape x *B. rapa* hybrid plants have been observed in the Netherlands.[8] Stable incorporation of genes from *B. napus* into wild *B. rapa* (introgression) has not been documented in the Netherlands, but has been reported in Canada.[9]

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 events MS8 and RF3 are produced by means of *Agrobacterium*-mediated transformation. Oilseed rape MS8xRF3 is produced by conventional crossbreeding of the parental oilseed rape events MS8 and RF3. Oilseed rape MS8, RF3 and MS8xRF3 express genes of a pollination control system, i.e. the *barnase* gene (present in MS8) which confers male sterility and the *barstar* gene (present in RF3) which restores male fertility. They also express the *bar* gene, which confers tolerance to glufosinate-ammonium containing herbicides.

Introduced genes	**Encoded proteins**	**Traits**
barnase (in RF3)	Encodes for the Barnase ribonuclease protein, which is expressed in the anther tapetal cell layer during pollen development.[10]	Confers male sterility.
barstar (in MS8)	Encodes for the tapetal-cell-specific Barstar ribonuclease-inhibitor protein.[11]	Restores male fertility.
bar (in RF3 and MS8)	Phosphinothricin N-acetyltransferase (PAT) enzyme. [12]	Tolerance to glufosinate-ammonium herbicides.
For a detailed description of the introduced genes and traits, see for example references 10 and 11		

3.3 Updated bioinformatics analyses and molecular characterisation

The application for the renewal of the authorisation for feed, import and processing of oilseed rape events MS8, RF3 and MS8xRF3 contains an updated molecular characterisation of the individual parental lines carried out using recent databases, which meet the requirements of COGEM.[13]

The insert and flanking regions in MS8 oilseed rape were originally sequenced in 2001. This sequence analysis revealed that the DNA sequence of the MS8 insert is completely identical to the corresponding plasmid T-DNA sequence. The insert and flanking regions in RF3 oilseed rape were also originally sequenced in 2001 and showed that RF3 oilseed rape contains two partial copies of T-DNA in the RF3 transgenic locus. Re-sequencing performed in 2013 confirmed all earlier results.

The molecular characterization performed in 2015 and 2016 confirmed that no endogenous genes were disrupted by the T-DNA insertions. The *in silico* analysis did not reveal sequence homology of the Barstar, Barnase and PAT proteins with other known proteins or toxins.

Conclusion: The molecular characterisation of MS8, RF3 and MS8xRF3 oilseed rape is adequate and no indications for potential environmental risks were identified.

3.4 Systemic literature search

The applicant performed a literature search using a broad collection of bibliographic databases, covering a publication period from May 2007 to May 2016. The search identified a total of 849 references, from which the applicant identified 6 as being relevant. According to the applicant these articles did not indicate any potential adverse effects on human and animal health.

According to Article 23 under the Regulation (EC) No 1829/2003 the applicant is obliged to provide all new information that has become available with regard to the evaluation of the safety in the use of the feed and risks of the feed to animals, humans or the environment. The systemic literature search performed by the applicant focussed on food and feed aspects and does not provide information on the potential environmental risks associated with MS8, RF3 and MS8xRF3 oilseed rape import and processing. This information is now part of the annual monitoring reports. For clarity purposes, COGEM is of the opinion that the systemic literature search can be improved by including the potential environmental risks associated with MS8, RF3 and MS8xRF3 import and processing.

Conclusion: The systemic literature search can be improved by including the potential environmental risks associated with MS8, RF3 and MS8xRF3 oilseed rape import and processing.

3.5 Annual monitoring reports

The applicant supplied annual monitoring reports carried out between May 2008 and July 2015. Monitoring was performed by operators involved in the import, handling and processing of viable oilseed rape RF3, MS8 and RF3xMS8; i.e., COCERAL, UNISTOCK and FEDIOL. Also, as part of the monitoring, the applicant performed a yearly review of scientific publications to monitor the safety of RF3, MS8 and RF3xMS8 oilseed rape. The monitoring results and literature search indicated no adverse health or environmental effects associated with the importation or use of MS8, RF3 and MS8xRF3 oilseed rape. However the occurrence of GM oilseed rape plants along import and transport routes and around processing plants has not been addressed in the annual monitoring reports (see more elaborate discussion in section 5).

Conclusion: The information in the annual monitoring reports gives no indication of adverse effects or incidents resulting from import and processing of MS8, RF3 and MS8xRF3 oilseed rape.

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)

COGEM has previously raised concerns with regard to PMEM of GM oilseed rape, COGEM is of the opinion that a more elaborate PMEM plan is needed. The applicant did not revise or update its current PMEM plan. As explained below, COGEM is of the opinion that the current PMEM plan does not meet the minimal requirements for feed, import and processing of GM oilseed rape.[4,5,6]

Feral MS8, RF3 and MS8xRF3 oilseed rape populations can arise at roadsides, railways and handling areas where spillage of seeds occurs during transshipment and transport. Plants arising from spilled MS8, RF3 and MS8xRF3 seeds will have a selective advantage over other plants in places where glufosinate-ammonium containing herbicides are used for weed control (for example along railways), because the use of these herbicides actively selects for herbicide tolerant MS8, RF3 and MS8xRF3 *B. napus*. In the long term, prolonged use of herbicides may lead to the establishment of feral herbicide tolerant GM *B. napus*, including plants with stacked events. This has been observed in *B. napus* cultivation areas in Canada and USA that are sprayed with herbicides.[4,5,6,14]

B. napus is able to cross-pollinate with its more common wild relative *B. rapa* and hybrid populations are present in the Netherlands.[6,15] Also, stable incorporation of transgenes from *B. napus* into wild *B. rapa* (introgression) has been reported.[9] Gene flow of GM traits could therefore potentially lead to incorporation of transgenes into the gene pool of *B. rapa* populations and lead to the establishment of feral herbicide tolerant *B. rapa* with GM traits.

It cannot be excluded that a possible combination of GM traits and/or a possible interaction between gene products, expressed by the genetic modification, may result in an adverse effect. In the view of COGEM, PMEM (i.e. general surveillance) is *the* instrument to identify such (in)direct, unanticipated, delayed, potentially adverse environmental effects.[4,5,6]

Based on these considerations, COGEM is of the opinion that the monitoring plan of MS8, RF3 and MS8xRF3 should include monitoring along transport routes (including roadsides and railway beddings) and transshipment areas. When GM oil seed rape is observed, *B. rapa* populations in the vicinity of the observed population should be monitored as well. This is a prerequisite to grant an authorisation for feed, import and processing of all GM oilseed rape events.[6] Therefore, COGEM is of the opinion that the PMEM plan of MS8, RF3 and MS8xRF3 needs to be adapted before a market authorisation for the renewal is granted.

6. Overall conclusion

There are no indications that expression of the introduced traits will alter the fitness of oilseed rape MS8, RF3 and MS8xRF3 under natural conditions. Import and processing of MS8, RF3 and MS8xRF3 pose a negligible risk in themselves to human health and the European environment. However, COGEM is of the opinion that the current monitoring plan is insufficient and should include monitoring along transport routes and transshipment areas. Therefore, COGEM cannot advise positively on the renewal application for feed, import and processing of MS8, RF3 and MS8xRF3 oilseed rape.

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