Application for import of MON88302 oilseed rape

COGEM advice CGM/120612-01

This advice concerns an application for import and processing of genetically modified MON88302 oilseed rape. MON88302 expresses a cp4 epsps *gene conferring tolerance to glyphosate containing herbicides.*

Oilseed rape has established itself in the Netherlands and is present across the country in small, local populations that are nearly always located on highly disturbed soil. Oilseed rape is present close to locations where seed spillage occurs or where oilseed rape is cultivated and does not establish well in existing vegetations.

*Oilseed rape is mainly a self-pollinating species, but outcrossing (30%) may occur. Controlled pollination studies have shown that oilseed rape can outcross with several of the wild relatives that occur in the Netherlands. Most hybrids have a severely reduced fertility. Exceptions are hybrids obtained from crosses between oilseed rape (*B. napus) *and turnip (*B. rapa).

The establishment of small populations of MON88302 oilseed rape on locations where glyphosate is frequently applied to control weeds e.g. on railway tracks, cannot be excluded. If small MON88302 populations would become established, these could cross-fertilise other oilseed rape plants and/or wild relatives, in particular turnip. Apart from the glyphosate tolerance trait, the resulting progeny will not possess a higher fitness and will not be different from progeny arising from cross-fertilisation with conventional oilseed rape varieties.

COGEM recommends including the monitoring of distribution routes for the occurrence of oilseed rape volunteers and populations in the monitoring plan. There is a small chance that cross-fertilisation could lead in the future to the stacking of several transgenes in a single oilseed rape plant. COGEM is of the opinion that stacking of the traits present in currently authorised oilseed rape varieties will not lead to an environmental risk because under natural conditions these traits are unlikely to lead to an increased fitness or a selective advantage. However, it is important to know whether stacked events arise in order to allow future risk assessments to take the presence of established GM oilseed rape with stacked traits into account. A stacked event would most likely occur in a location where herbicides are frequently used, such as railway tracks. Therefore, COGEM advises to involve railway companies and/or companies in charge of the maintenance of railway tracks in the post-market monitoring plan in order to monitor the occurrence of GM oilseed rape on railway tracks.

In conclusion, COGEM is of the opinion that import and processing of MON88302 oilseed rape poses a negligible risk to the environment. However, COGEM is of the opinion that the monitoring plan of MON88302 should be improved before a market authorisation is granted.

Introduction

The present application by Monsanto Europe S.A. (EFSA/GMO/BE/2011/101), concerns import and processing of genetically modified oilseed rape MON88302 and its use as any other conventional oilseed rape variety with the exception of cultivation. MON88302 expresses the *cp4 epsps* gene resulting in tolerance to glyphosate containing herbicides.

MON88302 oilseed rape has been submitted for regulatory approval concerning cultivation or import in several other countries beside the European Union, amongst others the USA, Canada, Japan, and the Philippines. The event has not yet received an authorisation for cultivation or import.

Import of a similar glyphosate tolerant oilseed rape event, GT73, was analysed by COGEM previously on several occasions.[1,2,3,4]

Considerations

Aspects of the crop

Oilseed rape (*Brassica napus*) is a member of the *Cruciferae* or *Brassicaceae* family, together with, among others, wild cabbage (*Brassica oleracea*), turnip *(Brassica rapa)* and black mustard (*Brassica nigra*).[5] *B. napus* (an allotetraploid with chromosome 2n = 38, AACC) originates from interspecific hybridization between the two diploid species *B. oleracea (2n* = 18, CC) and *B. rapa* (2n = 20, AA).[6]

Oilseed rape has been grown as a crop in the Netherlands since the early Middle Ages and is currently cultivated throughout the Netherlands.[6] Nowadays, oilseed rape cultivars usually produce seeds with a low glucosinolate and erucic acid content ("double low"). Cultivars with these characteristics are called canola. In addition, winter and spring varieties with differing vernalisation requirements are available for the different sowing seasons.

Oilseed rape reproduces sexually. It is mainly a self-pollinating species, but outcrossing may occur. In fields, the average rate of out-crossing between varieties of oilseed rape is 30%, but out-crossing rates between 12 to 55 % have been reported.[7,8] The pollen from oilseed rape can be transferred from plant to plant through physical contact between neighbouring plants and by wind and insects.[8,9] Spreading of oilseed rape pollen decreases rapidly with increasing distance from the source of the pollen. Long distance pollination events are presumably mediated by insects such as honeybees and bumblebees, which are attracted to the flowers of oilseed rape.[8,9]

Seeds of oilseed rape are small and produced in large quantities.[9] When seeds escape harvesting they can persist in the soil. Several publications on the persistence of oilseed rape seeds in the seed bank have been published. One of these publications describes a mean loss of 60% of the seeds in the first few months. The subsequent decline in seed number was observed to be much slower with a mean decline of 20% per year.[10] In another publication it was shown that only 1.5% of oilseed rape seeds survived the first year, and 0.2% survived the second year.[11] Under normal agricultural circumstances it was shown that oilseed rape seeds can persist for over four years,[10] and another study reported the occurrence of oilseed rape seedlings after a dormancy period of ten years.[12] These studies indicate that the seed bank has a quite rapid turnover, but that a small portion of oilseed rape seeds may remain viable for several years.

Oilseed rape has established itself in the Netherlands.[13] Recently, the assumption that oilseed rape is widespread in the Netherlands was refuted by results from a research project commissioned by COGEM. The researchers studied the distribution of oilseed rape in the Netherlands and found that oilseed rape (*B. napus*) is often confused with turnip (*B. rapa*). Oilseed rape was present across the Netherlands, but in general only a small number of plants (25 or less) was found on a single location and the presence of oilseed rape plants was often local.[6] Populations of oilseed rape are nearly always located on highly disturbed soil and close to locations where it is cultivated or where seeds are spilled in transport along roads or in transshipment.[6] These observations confirm previous reports that under non-cultivated conditions and without human intervention feral oilseed rape plants are mostly unable to survive for more than a few generations. Although some feral plants can persist for several years, there is no evidence that oilseed rape is invasive in undisturbed natural habitats.[15]

In the Netherlands the following wild relatives of oilseed rape (*B. napus*) are present: turnip (*B. rapa*), black mustard (*B. nigra*) and wild cabbage (*B. oleracea*). The latter is rare and only occurs in a few coastal areas.[5] Other closely related species that occur in the Netherlands are perennial wall rocket (*Diplotaxis tenuifolia*), shortpod mustard (*Hirschfeldia incana*), wild radish (*Raphanus raphanistrum*) and charlock (*Sinapis arvensis*).[5] Controlled pollination studies have pointed out that oilseed rape can outcross with several of the wild relatives that occur in the Netherlands. These relatives include *B. rapa*, *B. juncea*, *H. incana*, *R. raphanistrum* [9,14], *B. oleracea, S. arvensis*[14,15] and *D. tenuifolia*.[14] The potential for cross-pollination between oilseed rape and its wild relatives is influenced by several factors such as pollen viability, overlapping flowering period, distance, and insect activity.

Because of a mismatch in chromosome numbers most hybrids have a severely reduced fertility (very low pollen viability and seed production). Exceptions are hybrids obtained from crosses between oilseed rape and *B. rapa*. Only some of the interspecific embryos develop into viable seeds.[16] F1 hybrids resulting from these seeds can produce pollen and seed, although pollen viability and percentage seed set is typically lower than that of the parental species.[15] In the wild F1 hybrids between the two species are rare, but have been reported for Canada, the United States, the United Kingdom, Denmark, and the Netherlands.[17]

In a study focusing on hybridisation between oilseed rape and *B. rapa* in the Netherlands, Luijten & De Jong (2011) did not identify any wild *B. rapa* plants with additional chromosomes (i.e. C chromosomes from oilseed rape) nor did they find molecular markers characteristic for oilseed rape in *B. rapa* populations close to crops or feral populations of oilseed rape.[17] Therefore they argue that if hybrids between oilseed rape and *B. rapa* are formed, the progeny arising from crosses between these hybrids and *B. rapa* is probably unfit. This would limit the establishment of hybrids between oilseed rape and wild *B. rapa* to the first generation. Other researchers have also reported that backcross progeny of F1 hybrids exhibited a low survival rate and fertility.[16] Nevertheless, it has been shown that gene flow from oilseed rape to *B. rapa* can occur.[17,18]

Molecular characterisation
MON88302 was produced by *Rhizobium radiobacter* (formerly known as *Agrobacterium tumefaciens*) mediated transformation of conventional 'Ebony' oilseed rape hypocotyls using the PV-BNHT2672 plasmid.

An overview of the introduced sequences is given below:

- Right T-DNA border region from *R. radiobacter;*

- Synthetic intervening sequence (polylinker);
- P-*FMV/Tsf1*, chimeric promoter consisting of enhancer sequences from *Figwort mosaic virus* (FMV) *35S* gene combined with the promoter of the *Tsf1* gene of *Arabidopsis thaliana* encoding elongation factor EF-1α;
- L-*Tsf1*, 5' untranslated leader sequence from the *A. thaliana Tsf1* gene encoding elongation factor EF-1α;
- I- *Tsf1*, Intron from the *A. thaliana Tsf1* gene encoding elongation factor EF-1α;
- Synthetic intervening sequence (polylinker);
- TS-*CTP2*, N-terminal chloroplast transit peptide from the *epsps* gene of *A. thaliana;*
- CS-*cp4 epsps*, coding sequence for synthetic CP4 EPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase), originally from *Rhizobium radiobacter* strain CP4;
- Synthetic intervening sequence (polylinker);
- T-E9, 3' nontranslated region of the pea ribulose-1,5-bisphosphate carboxylase small subunit (*rbcS2*) E9 gene;
- Synthetic intervening sequence (polylinker);
- Left T-DNA border region from *R. radiobacter.*

Southern blot analysis indicated that no backbone elements of the plasmid were integrated. Southern blot analysis in combination with PCR analysis showed that MON88302 contains a single copy of the T-DNA insert. Sequence analysis of the T-DNA insert (4428 bp) showed that MON88302 contains the intact *cp4 epsps* expression cassette.

The regions flanking the insert were sequenced. The applicant used PCR analysis to verify that the regions flanking the insert of MON88302 are oilseed rape genomic DNA. A comparison of the sequences of the flanking region of the MON88302 insert and the sequence of the corresponding region in the non-transgenic oilseed rape variety indicated a few differences.

During the insertion, a deletion of 29 basepairs of the wild-type oilseed rape genome occurred. Immediately adjacent to the 3' end of the insert, MON88302 contains nine additional basepairs that are not present in this location in the wild-type genome. In addition, a one basepair change occurred within the 3' oilseed rape genomic DNA region. This difference was most likely caused by a single nucleotide polymorphism segregating in the oilseed rape population.

The flanking genomic DNA sequences were analysed for disruption of putative open reading frames (ORFs). No putative ORF was found spanning the insertion site, although the insertion site was found to be located in a region of about 90 nucleotides in between the 3' ends of two likely genes. There is no indication that these flanking genes are disrupted by the insertion of the T-DNA.

The junctions between the inserted sequences and the flanking regions of the oilseed rape genome were analysed to determine if new ORFs that putatively could produce novel chimeric proteins were created. The applicant selected eleven sequences spanning the junctions for analysis and deduced their amino acid sequences. None of these amino acid sequences showed homology to known allergens, toxins or other proteins.

COGEM notes that the applicant does not provide information on the criteria used for the selection of the ORFs spanning the junctions of the MON88302 T-DNA insert. However, from the provided sequences it can be deduced that ORFs putatively encoding eight or more amino acids were analysed from stop codon to stop codon. Therefore, COGEM is of the opinion that the molecular characterisation was adequately performed and meets the criteria as laid down by COGEM. [19]

Properties of the gene conferring glyphosate tolerance

MON88302 expresses the *cp4 epsps* gene from *R. radiobacter*. As a result, it produces the 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein.

EPSPS is a natural occurring enzyme involved in the biosynthesis of aromatic amino acids and is active in the chloroplasts of a plant cell. Glyphosate inhibits EPSPS, resulting in a lack of amino acids essential for growth and development of plants.[20,21] The CP4 EPSPS protein which is encoded by the *cp4 epsps* gene introduced in MON88302 has a reduced affinity for glyphosate. As a result MON88302 oilseed rape is tolerant to glyphosate containing herbicides.[21]

Environmental risk assessment

This application concerns an authorisation for import and processing of GM oilseed rape. Therefore, COGEM will only focus on the environmental risks associated with incidental spillage of oilseed rape.

Almost all import of oilseed rape seeds concerns the use of these seeds for oil production. In the Netherlands, all seed crushing industries are located near open water (sea, canal). The seeds are brought in by ships and transported to the crushing plant by road.[6] A small part of the imported oilseed rape seed is used in pet food, particularly for birds and rodents.[22]

The estimated seed loss during transport ranges from 0.1 to 3.0%.[17] Spillage of oilseed rape seeds during transport could lead to growth and establishment of oilseed rape. Oilseed rape populations usually consist of a small number of plants (25 or less) and are nearly always located on highly disturbed soil closely related to areas were oilseed rape is cultivated or where seed spillage occurred (along roads or in transhipment areas). Modern oilseed rape varieties, which have a low erucic acid content, have been found to form volunteer populations less often. Although some feral oilseed rape plants can persist for several years, there is no evidence that oilseed rape is invasive in undisturbed natural habitats.[15]

According to the applicant, MON88302 is not different from the conventional oilseed rape variety Ebony in terms of germination, vegetative and reproductive growth, yield, volunteers and susceptibility to insect, diseases and abiotic stresses. The studies supporting this claim did not show any indication that MON88302 has a higher fitness or an increased potential to form feral populations than conventional oilseed rape varieties.

MON88302 oilseed rape plants do not possess a selective advantage unless glyphosate is applied. In the Netherlands, the policy of the road maintenance authority is to use non-chemical methods to control weeds on the verges of roads.[23] Glyphosate application is however the most commonly used method of weed control along railway tracks in the Netherlands. Spilled GM oilseed rape may be able to form small volunteer populations along railway tracks where glyphosate is applied. These populations may be controlled by use of other herbicides, flame weeding or steam weeding. However, the establishment of small populations of spilled MON88302 oilseed rape cannot be excluded in disturbed environments where glyphosate is frequently applied.

If small MON88302 populations would become established, these could cross-fertilise other oilseed rape (*B. napus*) plants and/or wild relatives, in particular *B. rapa*. Apart from the glyphosate tolerance trait, the resulting progeny will not possess a higher fitness and will not be different from progeny arising from cross-fertilisation with conventional oilseed rape varieties.

In the European Union, a few transgenic oilseed rape varieties are authorised for import and processing. Because there is a small, but non-negligible possibility that cross fertilisation between *B. napus* plants arising from spilled seed could occur, there is a chance that several transgenes could be 'stacked' in a single oilseed rape plant.

The traits that have been introduced in the currently authorised transgenic oilseed rape varieties are tolerant to glufosinate ammonium containing herbicides and male sterility as well as the restoration of male sterility. COGEM is of the opinion that stacking of these traits in combination with glyphosate tolerance will not lead to an environmental risk, because under natural conditions these traits are unlikely to lead to an increased fitness or a selective advantage.

However, in view of future applications and to increase knowledge on the occurrence of stacked events it is important to know whether stacked events arise in order to allow future risk assessments to take the putative presence of established GM oilseed rape with stacked traits into account.

Incidental consumption

Since 2008 COGEM abstains from giving advice on the potential risks of incidental consumption in case a food/feed assessment is already carried out by other organisations.[24] This application is submitted under Regulation (EC) 1829/2003, therefore a food/feed assessment is carried out by EFSA. Other organisations who advise the competent authorities can perform an additional assessment on food safety although this is not obligatory. In the Netherlands a food and/or feed assessment for Regulation (EC) 1829/2003 applications is carried out by RIKILT. Regarding the risks for food and feed, the outcome of the assessment by other organisations (EFSA, RIKILT) was not known at the moment of the completion of this advice.

Post-market environmental monitoring

General surveillance has been introduced to be able to observe unexpected adverse effects of genetically modified (GM) crops on the environment. In general, the setting or population in which these effects might occur is either not, or hardly predictable. General surveillance for GT73 oilseed rape focuses on the import, handling and processing of viable GT73 oilseed rape.

Cross-fertilisation of GM oilseed rape volunteers could lead to stacking of different traits. Although COGEM does not consider stacking of traits from currently authorised GM oilseed rape varieties an environmental risk, it is important to know whether GM oilseed rape volunteers become established and stacked events arise from these populations. This will allow future risk assessments to take the putative presence of stacked oilseed rape events into account.

EFSA has stated in its guidance document that monitoring plans should address relevant exposure pathways.[25] Given the considerations above, COGEM points out that in the monitoring plan for MON88302 not all relevant exposure pathways for monitoring of GM oilseed rape are included. In 2010, COGEM remarked that general surveillance for crops that have outcrossing potential should cover handling areas and distribution routes.[26] In the Netherlands, populations of oilseed rape have been observed on several occasions near roads, railway tracks and railway stations.[6,22] Therefore, monitoring should pay special attention to these areas where viable oilseed rape seeds could be spilled unintentionally.

Also, since glyphosate application is the most common method of weed control along railway tracks in the Netherlands, railway companies and/or companies in charge of the maintenance of railway tracks (such as ProRail in the Netherlands) should be enlisted by the authorisation holder to monitor the occurrence of GM oilseed rape along railway tracks.

In 2010, COGEM formulated compliance criteria for General surveillance (GS) plans concerning Dutch applications for import and cultivation of GM crops.[26] In addition to the criteria mentioned above, the two following criteria are applicable to the GS plan of MON88302 oilseed rape.

In the EFSA guidance document, EFSA states that raw data and analysis of monitoring data should be made available by the applicant to the Competent Authorities and the European Commission.[25] COGEM agrees with this request and points out that the GS plan of MON88302 oilseed rape could be improved by a statement of the applicant on this point.[27]

The PMEM plan states that if the authorisation holder identifies an unexpected adverse effect caused by the GM plant, he will inform the European Commission immediately. COGEM is of the opinion that Member States should also be directly informed of these effects by the authorisation holder, to ensure that appropriate measures for protection of humans and the environment can be implemented immediately.

COGEM concludes that the GS plan for import and processing of MON88302 oilseed rape could be improved on several points. Most importantly, COGEM advises to include in the General surveillance plan that roadsides and railway beddings near oilseed rape transhipment and transport sites will be monitored for spillage of GM oilseed rape and stacking of event MON88302.

Conclusions and advice

Given the considerations above, COGEM is of the opinion that import and processing of MON88302 oilseed rape poses a negligible risk to the environment. However, COGEM advises to include in the PMEM plan that roadsides and railway beddings near oilseed rape transhipment and transport sites will be monitored for spillage of GM oilseed rape and stacking of event MON88302. COGEM is of the opinion that the PMEM plan for import and processing of MON88302 oilseed rape should be improved before a market authorisation for this event is granted.

Additional remarks

COGEM bases its deliberations on both the information and environmental risk analysis supplied by the applicant, as well as on any other relevant scientific knowledge and information concerning the topic of the application. Not all information in the dossier may be relevant for an environmental risk analysis as performed by COGEM. However, the submitted dossier should be of the highest quality. Unfortunately, this is not the case for the dossier supporting the application for import and processing of MON88302 oilseed rape.

For example, information on the criteria used by the applicant to select the ORFs spanning the insert-genomic DNA junctions for further analysis was absent in the application. To confirm whether the applicant selected the ORFs from stop to stop codon, the ORF sequences had to be studied in detail. COGEM points out that the information and experiments in the dossier should be presented in a clear and unambiguous manner, to avoid unnecessary confusion.

In addition, for the exposure characterisation, the applicant concludes that the exposure of organisms in the environment to MON88302 oilseed rape will be negligible. The applicant states that exposure is limited to accidental spillage of seed, to seed in faeces of animals fed MON88302 seed, and through organic plant matter. The applicant fails to take into account that oilseed rape may be used in bird seed and in garden flower seed. Also, the applicant argues that environmental conditions near spillage locations will not be conducive to germination, growth and reproduction of imported oilseed rape seeds. However, it is known from recent scientific literature that small oilseed rape volunteer populations may form after spillage. This is illustrated by the recent discovery of GT73 oilseed rape plants next to Swiss railways.[28] Therefore, the conclusion of the applicant concerning the exposure characterisation is not justified. For its own risk assessment, COGEM takes into account that spilled seed could produce volunteer populations.

COGEM remarks that the design of the agronomic field studies could be improved on several points, amongst others plot size, replication and statistical analysis of the results. Several parameters in the field trials resulted in discontinuous count data. Discontinuous count data has the property that the variance increases with the mean, which violates the Anova assumption of homogeneous variances. Anova is therefore not suitable as a tool for statistical analysis for these types of data. Also, an analysis of the statistical power of the test was not performed. The presented studies on dormancy do not address the occurrence of seed persistence in and outside the field. COGEM points out that to better support conclusions on ecological effects of genetic modification, like weediness potential, the design and statistical analysis of these experiments should be improved.

Since this application only concerns import and processing of oilseed rape, the trial designs are of lesser importance. However, this is a point of attention for possible future applications for cultivation of oilseed rape MON88302 in Europe or for import of oilseed rape events harbouring traits that effect plant physiology.

The above mentioned shortcomings do not affect the conclusions of the environmental risk assessment by COGEM. However, these flaws may be contra productive to the public trust in the risk assessment procedure.

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