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KENMERK CGM/130419-01
ONDERWERP Advies m.b.t. import en verwerking van gg- herbicidentolerant koolzaad
MS8xRF3xGT73

Geachte mevrouw Mansveld,

Naar aanleiding van een adviesvraag betreffende import en verwerking van de genetisch gemodificeerde herbicidentolerante koolzaadlijn MS8xRF3xGT73 van Bayer CropScience N.V. en Monsanto S.A. (EFSA/GMO/NL/2012/109), deelt de COGEM u het volgende mee.

Samenvatting

De COGEM is gevraagd te adviseren over een vergunningaanvraag voor import en verwerking van genetisch gemodificeerd (gg-)Koolzaad MS8xRF3xGT73. De koolzaadlijn is tolerant voor glyfosfaat en glufosinaat bevattende herbiciden en beschikt over een gecontroleerd bestuivingsmechanisme.

Door het morsen van zaden komen in Nederland koolzaadpopulaties voor langs transportroutes en bij overslagstations. Koolzaad kan kruisen met zijn wilde verwant Raapzaad.

De COGEM is van mening dat de moleculaire karakterisering van MS8xRF3xGT73 volledig is. Er zijn geen redenen om aan te nemen dat onder natuurlijke omstandigheden de expressie van de ingebrachte transgenen het verwilderingpotentieel van MS8xRF3xGT73 vergroot. Wel bieden de ingebrachte eigenschappen een selectief voordeel op locaties waar herbiciden worden gebruikt voor onkruidbestrijding, zoals langs spoorwegen.

De COGEM kan op voorhand niet uitsluiten dat er door uitkruising op termijn "stapeling" van transgene eigenschappen in koolzaadplanten kan optreden. Een mogelijke combinatie van transgene eigenschappen of een mogelijke interactie tussen producten van deze transgenen zouden tot een potentieel schadelijk milieu-effect kunnen leiden.

De COGEM beschouwt monitoring in de vorm van general surveillance (GS) het geëigende instrument om dergelijke indirecte potentieel schadelijke milieu-effecten te identificeren. Daarom acht zij het noodzakelijk dat er bij de importvergunning van gg-koolzaadlijn MS8xRF3xGT73 een monitoringsverplichting in de vorm van GS opgenomen wordt die zich richt op locaties waar de kans het grootst is wilde gg-koolzaadpopulaties aan te treffen, zoals langs spoorwegen. Indien gg-koolzaad wordt waargenomen, dient er ook monitoring van in de buurt liggende raapzaadpopulaties plaats te vinden op de aanwezigheid van transgene eigenschappen. De COGEM is van mening dat het monitoringsplan van MS8xRF3xGT73 verbeterd moet worden alvorens er een markttoelating voor import van deze koolzaadlijn afgegeven wordt.

De door de COGEM gehanteerde overwegingen en het hieruit voortvloeiende advies treft u hierbij aan als bijlage.

Hoogachtend,



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Advisory report on import of herbicide tolerant oilseed rape MS8xRF3xGT73

COGEM advisory report CGM/130419-01

This advisory report concerns an application for import and processing of genetically modified (GM) oilseed rape (Brassica napus) MS8xRF3xGT73. MS8xRF3xGT73 is tolerant for glufosinate ammonium containing herbicides, tolerant and resistant for glyphosate containing herbicides and expresses a pollination control system.

COGEM is of the opinion that the molecular characterisation of MS8xRF3xGT73 has been performed adequately. The environmental risk assessment of MS8xRF3xGT73 indicates that the event in itself does not pose a risk to the environment in the Netherlands. However, COGEM is of the opinion that the provided general surveillance plan does not meet the minimal requirements for import and processing of MS8xRF3xGT73.

The establishment of small populations of MS8xRF3xGT73 oilseed rape cannot be excluded on locations where glyphosate or glufosinate ammonium are frequently applied to control weeds. Further cross-fertilisation can eventually lead to stacking of several transgenes in one plant. This phenomenon has been reported along roadsides in North America which had been sprayed with herbicides.

Furthermore, interspecific hybridisation between oilseed rape and its wild close relative Brassica rapa occurs under natural conditions and has been reported in the Netherlands. It is known that backcrosses between these hybrids and B. rapa may occur in the field.

If stacking of transgenes occurs in the wild, it cannot be excluded that a possible new combination of GM traits and/or a possible interaction between their genes may result in a potentially adverse effect. Therefore, general surveillance of feral GM oilseed rape populations will be needed to identify in time such unanticipated, delayed or (in)direct adverse environmental effects. As these (stacked) GM oilseed rape plants are most likely to occur in handling areas and along transport routes, in particular when herbicides are used for weed control such as on railway tracks, general surveillance should focus on these areas. Where GM oilseed rape plants occur, transgene flow to B. rapa is possible. Any B. rapa populations in the vicinity of GM oilseed rape should therefore be included in the general surveillance plan and monitored for the presence of transgenes.

In summary, COGEM is of the opinion that the monitoring plan of MS8xRF3xGT73 should be improved before a market authorisation is granted. Therefore, COGEM cannot advise positively on the application for import and processing of MS8xRF3xGT73 oilseed rape.

1. Introduction

The present application by Monsanto Company and Bayer CropScience AG (EFSA/GMO/NL/2009/75) concerns import and processing of genetically modified (GM) oilseed rape MS8xRF3xGT73 and its use as any other conventional oilseed rape variety with the exception of cultivation. GM oilseed rape MS8xRF3xGT73 is tolerant for certain herbicides and expresses a pollination control system.

In 2011, MS8xRF3xGT73 oilseed rape was approved for food applications in Mexico. In 2012, MS8xRF3xGT73 oilseed rape was approved for cultivation, import and processing in Japan.¹ The parental lines GT73, Ms8, Rf3 and Ms8xRF3 have been approved for cultivation, import and processing in Australia, Canada, Japan and the USA.^{1,2} Additionally the parental

lines have been approved for import and processing as food and/or feed in the European Union (EU), China, the Philippines, South-Korea, Mexico, New Zealand and South Africa.^{1,2}

2. Previous COGEM advisory reports

In 2004, COGEM advised positively on import, processing and cultivation of oilseed rape events MS8, RF3 and MS8xRF3.³ In 2009, COGEM concluded that an update of the molecular characterisation of RF3 was adequate and fulfilled the criteria of COGEM.⁴ COGEM issued several advisory reports concerning the import and processing of oilseed rape event GT73,^{5,6,7,8,9,10} and concluded that the post-market environmental monitoring (PMEM) plan of this event did not fulfill the criteria of COGEM.^{9,10}

3. Aspects of the crop

Recently, COGEM issued an advisory report on the aspects relevant for the environmental risk assessment (ERA) of import and processing of GM oilseed rape (*Brassica napus*) in the Netherlands.¹¹ In the advisory report, the biological characteristics of the crop have been extensively discussed. A summary of these biological characteristics is outlined below.

Oilseed rape is an important oil plant cultivated in the temperate climate regions of the world. The plant is able to form volunteers in disturbed 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 along distribution routes and handling areas. Population sizes vary from a few individuals to several hundreds of plants. Oilseed rape seeds generally display little dormancy. The seed bank of oilseed rape has quite a rapid turnover but a small portion of the seeds may remain viable for over four years. The occurrence of seedlings after a dormancy period of 10 years has been reported.

Oilseed rape can cross-pollinate with wild relatives. Controlled pollination experiments have shown that the species can fertilise several allied genera. Most interspecific hybrids have a severely reduced fertility (very low pollen viability and seed production). Exceptions are hybrids obtained from crosses between oilseed rape and *Brassica rapa*, and to a lesser extent oilseed rape x *Brassica juncea* and oilseed rape x *Brassica oleracea*.

If feral oilseed rape populations establishes, both intraspecific and interspecific flow of (trans)genes have been reported under naturally occurring conditions. Oilseed rape x *B. rapa* hybrids have been observed at locations where both species co-occur.

If herbicide tolerant GM oilseed rape is imported or cultivated, herbicide tolerant populations can occur along transport routes. Herbicide use actively selects for herbicide tolerant plants. The unintentional stacking of herbicide tolerance traits in oilseed rape has been observed under natural conditions in regions where GM oilseed rape is imported as well as in GM oilseed rape cultivation areas.^{12,13,14,15}

In the Netherlands, *B. rapa* is more common than oilseed rape. *B. rapa* is found in similar areas, such as disturbed habitats in road verges and waste grounds.¹⁶ If *B. rapa* populations exist in close proximity of feral oilseed rape plants, feral oilseed rape x *B. rapa* hybrids may arise by hybridisation, as has been reported in several countries. Occasionally, oilseed rape x *B. rapa* hybrids of the first generation have been observed in the Netherlands.¹⁷ Transgene flow from oilseed rape to *B. rapa* has been reported in natural conditions.¹⁸ Additionally, back-cross progeny and the stable incorporation of a herbicide tolerance transgene into a *B. rapa* plant have been observed in the wild.

In summary, feral (GM) oilseed rape populations can persist for several years. Intraspecific (trans)gene flow and interspecific (trans)gene flow between oilseed rape and *B. rapa* have been observed. Stacking of transgenes into feral oilseed rape and introgression of transgenes into feral *B. rapa* occur under natural conditions.

4. Points to consider for import and processing of MS8xRF3xGT73

4.1 Properties of the introduced transgenes conferring herbicide tolerance

In non-transgenic plants the herbicide glufosinate ammonium inhibits the activity of glutamine synthetase, an enzyme necessary for the production of the amino acid glutamine and for ammonia detoxification.¹⁹ As a result, glutamine levels are reduced and ammonia levels are increased. Photosynthesis is inhibited and eventually the plant dies.²⁰ Glyphosate containing herbicides inhibit 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). EPSPS is a natural occurring enzyme involved in the biosynthesis of aromatic amino acids and is active in the chloroplasts of a plant cell. As a result, the plant lacks amino acids essential for growth and development.^{21,22}

Stacked event GM oilseed rape MS8xRF3xGT73 expresses the *bar* gene (originating from parental lines MS8 and RF3)³, which encodes phosphinothricin-N-acetyl transferase (PAT). The PAT enzyme acetylates L-phosphinothricin, the active isomer of glufosinate ammonium. The resulting compound N-acetyl-L-phosphinothricin does not inhibit the activity of glutamine synthetase.¹⁹ As a result MS8xRF3xGT73 is tolerant to L-phosphinothricin and thus to herbicides containing glufosinate ammonium.

Additionally, MS8xRF3xGT73 expresses the *cp4 epsps* and *goxv247* genes (originating from parental line GT73)⁸. The CP4 EPSPS protein has a reduced affinity for glyphosate. Glyphosate oxidoreductase (GOX) acts by breaking down glyphosate into aminomethylphosphonic acid (AMPA) and glyoxylate.²³ The GOXv247 protein is a modified GOX protein, which increases the efficiency of glyphosate degradation. As a result, MS8xRF3xGT73 is tolerant and resistant to glyphosate.^{22,23}

4.2 Properties of the introduced transgenes conferring a pollination control system

MS8xRF3xGT73 expresses the barnase gene (originating from MS8) and barstar gene (originating from RF3), conferring male sterility and fertility restoration respectively. The genetic modification results in a plant with a pollination control system.²⁴

4.3 Molecular characterisation

Oilseed rape MS8xRF3xGT73 was produced by conventional crossbreeding of the three GM parental oilseed rape events MS8, RF3 and GT73. COGEM evaluated the molecular characterisation of the parental lines in previous applications concerning import and processing, and concluded that the molecular characterisation of the individual parental lines was adequate.^{3,4,7,8}

4.4 Environmental risk assessment

In the EU, authorisation of a GM crop is subject to the outcome of an ERA. The purpose of the ERA is to assess whether the introduction of the GM plant into the environment would have adverse effects on human and animal health and on the environment. Relevant points to consider are, amongst other things, the capability of the GM crop to form feral populations,

and its weediness and outcrossing potential. Several biological characteristics are taken into account such as vegetative and reproductive growth, seed germination, dormancy, seed bank formation, persistence, fitness (susceptibility to insects, diseases and abiotic stresses) and hybridisation with closely related species.

This application concerns the authorisation for import and processing of GM oilseed rape MS8xRF3xGT73. Therefore, COGEM will only focus on the environmental risks associated with incidental seed spillage of this GM event.

There is little evidence that oilseed rape is invasive although the crop is able to form feral populations, which can persist for several years. Due to seed spillage, feral populations occur along distribution routes and transshipment areas (reported in both the Netherlands and other countries).¹¹ The GM traits in the stacked event MS8xRF3xGT73 include tolerance and resistance to the herbicide glyphosate, tolerance to the herbicide glufosinate ammonium and a pollination control system. Herbicide tolerance can confer a selective advantage when the herbicide is applied, as observed in several countries.^{25,26} In the Netherlands, the policy of the road maintenance authority is to use non-chemical methods to control weeds on road verges. However, along railways glyphosate and to a lesser extent glufosinate applications are the most commonly used methods of weed control.²⁷

COGEM points out that spilled herbicide tolerant GM oilseed rape seeds will have a selective advantage over other plants if herbicides are used for weed control and GM herbicide tolerant feral oilseed rape populations may occur. Under natural conditions, the GM traits in MS8xRF3xGT73 themselves, the interactions between its transgenes and the interactions between their gene products are unlikely to lead to an increased fitness or a selective advantage. Therefore, in the view of COGEM import and processing of stacked event MS8xRF3xGT73 in itself will not lead to an environmental adverse effect.

4.5 Post-market environmental monitoring

Holders of GM crop authorisations are required to monitor for the occurrence of adverse effects from the import and/or cultivation of the GM crop.²⁸ PMEM consists of two parts: 'case-specific monitoring' and 'general surveillance'.

Case-specific monitoring is designed to confirm that any hypotheses regarding the occurrence and impact of potentially adverse effects of the GM crop or its use in the ERA are correct. Case-specific monitoring is therefore only necessary when the ERA identifies a potentially adverse effect to the environment.

As stated in the EFSA guidance on the PMEM of GM plants (*Legislative Background*), general surveillance is instituted 'in order to trace and identify any direct or indirect, immediate, delayed or unanticipated effects on human health or the environment of GMOs as or in products after they have been placed on the market'.²⁸ General surveillance is *the* instrument 'to identify the occurrence of adverse effects of the GMO or its use on human health or the environment which were not anticipated in the ERA'.²⁸ If an adverse effect is identified, remedial measures can be taken. The preparation and implementation of a general surveillance plan is compulsory for all applications of GM crops.

4.5.1 Case-specific monitoring

Taking into consideration the afore-mentioned considerations regarding the ERA (*section 4.4*), in the view of COGEM case-specific monitoring of MS8xRF3xGT73 is not necessary

because the ERA has not identified potentially adverse effects on the environment by the oilseed rape event itself.

4.5.2 General surveillance

MS8xRF3xGT73 populations can arise at roadsides, railways and handling areas where spillage of seeds occurs during transshipment and transport. As pointed out before (section 4.4), spilled MS8xRF3xGT73 seeds will have a selective advantage over other plants if herbicides are used for weed control and, consequently, feral MS8xRF3xGT73 populations may occur. There is a chance that intraspecific transgene flow by cross-fertilisation from feral GM oilseed rape plants harbouring other GM traits, may lead to the additional stacking of transgenes in one oilseed rape plant. The stacking of these different events may result in feral oilseed rape populations with new GM trait combinations, which have not yet been assessed. Interactions between these new combinations of transgenes and/or transgene products may lead to a potentially long-term adverse environmental effect. Additionally, gene flow of MS8xRF3xGT73 GM traits could lead to the incorporation of one or more transgenes into the gene pool of *B. rapa* populations. The introduction into the environment of *B. rapa* plants with GM traits have not yet been assessed.

As pointed out before (section 4.5.2), general surveillance is used to identify any unexpected, (in)direct, (possibly adverse) effects of GM populations on the environment. In the view of COGEM monitoring should take place where the occurrence of feral GM populations is possible, enabling the identification of any environmental effect, whether it is adverse or not. In 2010, COGEM remarked in a topic report that general surveillance for import applications of GM crops, which have out-crossing potential (such as oilseed rape), should generally cover handling areas and distribution routes.²⁹

Since it cannot be excluded that under selective herbicidal pressure GM oilseed rape MS8xRF3xGT73 may form feral populations and may cross-fertilise oilseed rape plants harbouring different GM traits, the possible new combination of stacked transgenes and/or a possible interaction between their gene products, may result in a potentially adverse environmental effect. In the view of COGEM, general surveillance is *the* instrument to identify such (in)direct, unanticipated, delayed, potentially adverse environmental effects. Therefore, in the general surveillance plan monitoring of MS8xRF3xGT73 transport routes (including roadsides and railway beddings) and transshipment areas should be included. If MS8xRF3xGT73 is observed, *B. rapa* populations in the vicinity of the observed MS8xRF3xGT73 population will have to be included in the general surveillance plan as well.

Since glyphosate application is the most common method for weed control along railways in the Netherlands, railway companies and/or companies in charge of the maintenance of railways should be involved by the authorisation holder in monitoring for the occurrence of MS8xRF3xGT73 and *B. rapa* with GM traits along railways. As stated in the earlier mentioned topic report on general surveillance, COGEM is of the opinion that it is important to call in (floristically) qualified existing monitoring networks.²⁹

COGEM has several additional comments on the general surveillance plan of import and processing of oilseed rape MS8xRF3xGT73. EFSA has stated in its guidance document that raw data and analysis of monitoring data should be made available by the applicant to the Competent Authorities and the European Commission.³⁰ COGEM agrees with this request

and points out that the GS plan of MS8xRF3xGT73 oilseed rape could be improved by a statement of the applicant on this point.³¹

In the present GS plan, the authorisation holder states that the operators have agreed to provide information relevant to the monitoring of MS8xRF3xGT73 to the authorisation holder. The GS plan could be improved by a guarantee that operators will monitor for unanticipated effects. This is in conformity with the criteria laid down by COGEM.²⁹

The GS 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 PMEM plan for import and processing of MS8xRF3xGT73 oilseed rape should be improved on several points. Most importantly, COGEM advises to include monitoring of roadsides and railway beddings near oilseed rape transshipment and transport sites for spillage of GM oilseed rape and stacking of event MS8xRF3xGT73 oilseed rape.

4.6 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.³² 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.

5. Conclusion

In summary, COGEM is of the opinion that the PMEM concerning import and processing of oilseed rape MS8xRF3xGT73 could be improved on several points. Most importantly, the approval of the application should depend on the inclusion of monitoring along transport routes or transshipment areas in the general surveillance plan. Special attention should be paid to the areas where viable MS8xRF3xGT73 oilseed rape seeds could be spilled unintentionally, enabling the identification of any direct or indirect, immediate, delayed or unanticipated environmental effects.

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