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KENMERK CGM/120203-01
ONDERWERP Advies m.b.t. import en verwerking van koolzaad GT73 met glyfosaat-tolerantie en resistentie

Geachte heer Atsma,

Naar aanleiding van een adviesvraag betreffende import en verwerking van de genetisch gemodificeerde (gg-) glyfosaattolerante en resistente koolzaadlijn GT73 van Monsanto Europe S.A. (EFSA/GMO/NL/2010/87), deelt de COGEM u het volgende mee.

Samenvatting
De COGEM is gevraagd te adviseren over een vergunningaanvraag voor import en verwerking van gg-koolzaad GT73. Deze koolzaadlijn brengt de genen *goxv247* en *cp4 epsps* tot expressie en is als gevolg hiervan tolerant voor en resistent tegen glyfosaat bevattende herbiciden.
Koolzaad kan zich in Nederland vestigen op plaatsen waar de bodem is verstoord. In Nederland komen verschillende verwante soorten voor waarmee koolzaad kan kruisen, zoals raapzaad. Er kan niet uitgesloten worden dat bij het incidenteel morsen van GT73 koolzaad een kleine populatie GT73 koolzaad ontstaat en dat GT73 andere koolzaadplanten of wilde verwante soorten bevrucht. Met uitzondering van de tolerantie voor glyfosaat verschilt GT73 niet van conventionele koolzaadrassen. De in GT73 ingebrachte eigenschappen bieden alleen een selectief voordeel op plaatsen waar vooral glyfosaat wordt gebruikt, zoals langs spoorwegen.
De moleculaire karakterisering van GT73 voldoet aan alle eisen van de COGEM. De resultaten hiervan geven geen reden om nadelige effecten te verwachten.
In de vergunningaanvraag ontbreekt echter een ‘post-market environmental monitoring plan’. De vergunningaanvraag is daardoor onvolledig.
De COGEM adviseert om te monitoren op de aanwezigheid van gg-koolzaad en om spoorwegbedrijven en/of instellingen die het onderhoud van spoorwegen verzorgen, hierbij in te schakelen. Gemorst GT73 koolzaad kan mogelijk met andere koolzaadplanten kruisen waardoor verschillende transgene eigenschappen gecombineerd zouden kunnen worden. De COGEM is van mening dat dit bij de huidige toegelaten koolzaadlijnen niet tot een milieurisico zal leiden, maar adviseert om met het oog op potentiële nieuwe vergunningaanvragen de aanwezigheid van gg-koolzaad langs spoorwegen te monitoren, zodat bij toekomstige vergunningaanvragen met eventueel gevestigd gg-koolzaad rekening gehouden kan worden.
Concluderend acht de COGEM de milieurisico’s van import van GT73 gezien de huidige omstandigheden verwaarloosbaar klein, maar kan zij gezien de onvolledige vergunningaanvraag geen eindoordeel geven over deze vergunningaanvraag.

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

Hoogachtend,

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Voorzitter COGEM

c.c. Drs. H.P. de Wijs
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Advice on import and processing of GT73 oilseed rape
COGEM advice CGM/120203-01

This advice concerns an application for import and processing of genetically modified GT73 oilseed rape. GT73 expresses the goxv247 *and* cp4 epsps *genes conferring tolerance and resistance 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 pointed out 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* B. rapa.

*The establishment of small populations of GT73 oilseed rape on locations where glyphosate is frequently applied to control weeds e.g. on railway tracks, cannot be excluded. Apart from the tolerance to glyphosate containing herbicides, GT73 does not differ from conventional oilseed rape. If small GT73 populations would become established, these could cross-fertilize other oilseed rape (*B. napus*) plants and/or wild relatives, in particular* B. rapa. *Apart from the glyphosate tolerance trait, the resulting progeny does not possess a higher fitness and is not different from progeny arising from cross-fertilization with conventional oilseed rape varieties.*

The molecular characterization of GT73 does not give any reason to expect adverse effects. It is adequately performed and meets the criteria laid down by COGEM.

However, since the application does not contain a post-market monitoring plan COGEM considers the application to be incomplete.

COGEM recommends involving 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 along railway tracks. There is a small chance that cross-fertilizaton 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 authorized 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, in view of future applications and to increase knowledge 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. A putative stacked event would most likely occur in a location where herbicides are frequently used, such as railway tracks. Therefore, COGEM advises to monitor the occurrence of GM oilseed rape along railway tracks in addition to the monitoring of industrial sites that is usually carried out by operators involved in import and processing of GM crops.

In conclusion, although COGEM is of the opinion that in view of the present conditions import and processing of GT73 oilseed rape poses a negligible risk to the environment, COGEM cannot finalize its opinion on import and processing of GT73 due to the absence of a post-market monitoring plan.

Introduction

The present application by Monsanto Europe S.A. (EFSA/GMO/NL/2010/87), concerns import and processing of genetically modified oilseed rape GT73 and its use as any other conventional oilseed rape variety with the exception of cultivation. GT73 expresses the *goxv247* and *cp4 epsps* genes resulting in tolerance and resistance to glyphosate containing herbicides.

COGEM has previously issued several advices concerning import and processing of GT73 oilseed rape.[1,2,3] In its latest advice, COGEM concluded that import and processing of GT73 poses a negligible risk to the environment.[3]

In 2004 and 2009, EFSA concluded that GT73 oilseed rape is unlikely to have an adverse effect on human and animal health and the environment in the context of its proposed uses i.e. import and processing, and the continued marketing of existing food and feed produced from GT73.[4,5] In 2005, GT73 was authorized in the European Union for the same uses as conventional oilseed rape with the exception of cultivation and uses as or in food.[6]

Recently, Monsanto submitted an additional application for authorization of GT73, because the company wanted to ensure that in the European Union the entire range of uses of GT73 oilseed rape (with the exception of cultivation), is authorised.

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*).[7] *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).[8]

Oilseed rape has been grown as a crop in the Netherlands since the early Middle Ages and is currently cultivated throughout the Netherlands.[8] Nowadays, oilseed rape cultivars usually produce seeds with a low glucosinolate and erucic acid content ("double low"). Cultivars with these characteristics are called canola.

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.[9,10] The pollen from oilseed rape can be transferred from plant to plant through physical contact between neighbouring plants and by wind and insects.[10,11] 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.[10,11]

Seeds of oilseed rape are small and produced in large quantities.[11] When seeds escape harvesting they can persist in the soil. Several publications on the persistence of oilseed rape seeds in the seedbank were published. One of these publications described 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.[12] 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.[13] Under normal agricultural circumstances it was shown that oilseed rape seeds could persist for over four years,[12] and another study reported the occurrence of oilseed rape seedlings after a dormancy period of ten years.[14] These studies indicate that the seedbank 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.[15] In the past, it was assumed that oilseed rape was widespread in the Netherlands. Oilseed rape (*B. napus*) is, however, often confused with *B. rapa*. To gain more insight in the distribution of oilseed rape in the Netherlands COGEM commissioned a research project. The results from this project showed that oilseed rape was present across the Netherlands, but that usually only a small number of plants (25 or less) was found on a single location and that the presence of oilseed rape plants was often local.[8] Populations of oilseed rape are nearly always located on highly disturbed soil and close to locations where it is cultivated or seeds are spilled in transport along roads or in transshipment.[8] These observations confirm previous reports that under non-cultivated conditions and without human intervention feral oilseed rape plants are usually 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.[17]

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.[7] 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*).[7] 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* [11,16], *B. oleracea*, *S. arvensis*[16,17] and *D. tenuifolia*.[16] 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 (*B. napus*) and *B. rapa.* Although only some of the interspecific embryos develop into viable seeds,[18] any F1 hybrids resulting from these seeds grow vigorously and can produce pollen and seed, though pollen viability and percentage seed set is typically lower than that of the parental species.[17] 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.[19]

Luijten & De Jong (2011) did not identify any *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 in the Netherlands. Therefore they argue that any progeny arising from crosses between F1 hybrids and *B. rapa* is probably unfit. Other researchers have also reported that backcross progeny of F1 hybrids exhibited a low survival rate and fertility.[18] Nevertheless, it has been shown that gene flow from oilseed rape (*B. napus*) to *B. rapa* can occur.[19,20]

Molecular characterization

GT73 was produced by *Agrobacterium tumefaciens* mediated transformation of oilseed rape using the PV-BNGT04 plasmid.

An overview of the introduced sequences is given below:

- Right T-DNA border region from *A. tumefaciens;*
- Synthetic intervening sequence (polylinker);
- P-FMV, 35S promoter from a modified *Figwort Mosaic Virus* (FMV);
- Synthetic intervening sequence (polylinker);
- TS-CTP1, N-terminal chloroplast transit peptide from the small subunit 1A of the ribulose-1,5-bisphosphate carboxylase gene from *Arabidopsis thaliana;*
- CS-*goxv247*, synthetic glyphosate oxidoreductase (*gox*) gene variant of the glyphosate oxidoreductase gene from *Ochrobactrum antrhopi* (formerly classified as *Achromobacter* sp.) strain LBAA;
- Synthetic intervening sequence (polylinker);
- Synthetic intervening sequence (polylinker);
- T-E9, 3' nontranslated region of the pea ribulose-1,5-bisphosphate carboxylase small subunit (*rbcS*) E9 gene;
- Synthetic intervening sequence (polylinker);
- P-FMV, 35S promoter from a modified FMV;
- TS-CTP2, N-terminal chloroplast transit peptide from the *epsps* gene of *A. thaliana ;*
- CS-*cp4 epsps*, synthetic CP4 EPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase) from *Agrobacterium* sp. strain CP4;
- Synthetic intervening sequence (polylinker);
- T-E9, 3' nontranslated region of the pea ribulose-1,5-bisphosphate carboxylase small subunit (*rbcS*) E9 gene;
- Synthetic intervening sequence (polylinker);
- Left T-DNA border region from *A. tumefaciens.*

Southern blot analysis indicated that no backbone elements of the plasmid were integrated in GT73. Southern blot analysis in combination with PCR analysis showed that GT73 contains a single copy of the T-DNA insert. Sequence analysis of the T-DNA insert (6,238 bp) showed that GT73 contains intact expression cassettes of *goxv247* and *cp4 epsps*.

The regions flanking the insert were partly sequenced (146 bp into the 5' flanking region and 238 bp into the 3' flanking region). The applicant used PCR analysis to verify that the regions flanking the insert of GT73 are oilseed rape genomic DNA.

A comparison of the sequences of the flanking region of the GT73 insert and the sequence of the corresponding region in the non-transgenic oilseed rape variety indicates that 40 basepairs of the wild-type oilseed rape genome are absent in GT73. In addition, immediately adjacent to the 5' end of the insert, GT73 contains 22 additional basepairs that are not present in this location in the wild-type oilseed rape genome. As a result, the 5' end of the insert in GT73 contains two junctions.

The three junctions between the inserted sequences and the flanking regions of the oilseed rape genome were analysed to determine if new open reading frames (ORFs) that putatively could produce novel chimeric proteins were created. ORFs putatively encoding 8 or more amino acids were analysed from stop codon to stop codon and their amino acid sequences were deduced. In total, 13 sequences were analysed, because one of the possible frames contained two putative new ORFs (one originating in the insert and terminating in the 22 basepairs fragment and the second originating in the 22 basepairs fragment and terminating in the 5’ flanking region). None of the deduced amino acid sequences show homology to known allergens, toxins or other proteins.

COGEM is of the opinion that the molecular characterization of GT73 was adequately performed and meets the criteria laid down by COGEM.[21]

Properties of the genes conferring glyphosate tolerance and resistance

GT73 expresses the *cp4 epsps* and *goxv247* genes. As a result it produces the 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) and the glyphosate oxidoreductase (GOX) proteins. These proteins confer tolerance and resistance to glyphosate.

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.[22,23] The CP4 EPSPS protein which is encoded by the *cp4 epsps* gene introduced in GT73 has a reduced affinity for glyphosate. As a result GT73 oilseed rape is tolerant to glyphosate containing herbicides.[23]

Glyphosate oxidoreductase (GOX) acts by breaking down glyphosate into aminomethylphosphonic acid (AMPA) and glyoxylate.[24] The GOXv247 protein which is produced by GT73 was modified (3 out of 431 amino acids were changed) to increase the efficiency of glyphosate degradation, thus allowing GT73 plants to grow when treated with glyphosate.

Environmental risk assessment

This application concerns an authorization 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.[8] A small part of the imported oilseed rape is used in pet food, particularly for birds and rodents.[25]

The estimated seed loss during transport ranges from 0.1 to 3.0%.[19] 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 transshipment areas). Although some feral plants can persist for several years, there is no evidence that oilseed rape is invasive in undisturbed natural habitats.[17]

According to the applicant, GT73 is not different from the conventional oilseed rape variety Westar 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 GT73 has a higher fitness or an increased potential to form feral populations than conventional oilseed rape varieties.

GT73 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.[26] Glyphosate may, however, be used to control weeds in other locations such as railroads.

In conclusion, the establishment of small populations of GT73 oilseed rape on locations where glyphosate is frequently applied e.g. on railway tracks, cannot be excluded. Apart from the tolerance to glyphosate containing herbicides, GT73 does not differ from conventional oilseed rape.

If small GT73 populations would become established, these could cross-fertilize other oilseed rape (*B. napus*) plants and/or wild relatives, in particular *B. rapa*. Apart from the glyphosate tolerance trait, the resulting progeny does not possess a higher fitness and is not different from progeny arising from cross-fertilization with conventional oilseed rape varieties.

In the European Union, a few transgenic oilseed rape varieties are authorized for import and processing. Because there is a small, but non-negligible possibility that cross fertilization 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 authorized 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. A putative stacked event would most likely occur in a location where herbicides are frequently used, such as railway tracks. Therefore, COGEM recommends involving railway companies and/or companies in charge of the maintenance of railway tracks (such as ProRail in the Netherlands) in the post-market monitoring plan in order to monitor the occurrence of GM oilseed rape along railway tracks.

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.[27] 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

COGEM points out that no post-market environmental monitoring plan was included in this application (see additional remark-1). Therefore, COGEM is of the opinion that at this moment the application is incomplete.

In COGEM's view, one of the points that should be included in the post-market monitoring plan is the involvement of railway companies and/or companies in charge of the maintenance of railway tracks (such as ProRail in the Netherlands) to monitor the occurrence of GM oilseed rape along railway tracks.

Conclusions and advice

In view of the aforementioned, COGEM is of the opinion that under the present conditions import and processing of GT73 oilseed rape poses a negligible risk to the environment. However, due to the absence of a post-market monitoring plan COGEM cannot finalize its opinion on import and processing of GT73.

In view of putative future authorizations COGEM advises to monitor the occurrence of GM oilseed rape along railway tracks in addition to the monitoring of industrial sites that is usually carried out by operators involved in import and processing of GM crops.

Additional remark

COGEM notes that there appears to be an inconsistency between EFSA and the applicant with regard to the scope of this application. In the application it is mentioned that the scope of the application concerns food containing and consisting of GT73 oilseed rape; and food produced from or containing ingredients produced from GT73, with the exception of refined oil and food additives. It is also mentioned that import, storage and processing of viable grains of GT73 are not in the scope of the application. In addition, the absence of a post-market environmental monitoring plan and the statement of the applicant that in this case an environmental risk assessment (ERA) is not required, seem to support that an authorization for viable material is not part of the current application.

EFSA, however, invited the national competent authorities within the meaning of Directive 2001/18/EC to submit their comments to EFSA, thus suggesting that the application did include viable material. On inquiry EFSA confirmed that viable grains were included in the scope of the application.

Therefore, there appears to be a contradiction between the applicant and the EFSA with regard to the exact scope of the application. COGEM points out that a clear, unequivocal scope is crucial for the risk assessment process. In view of the current uncertainties with regard to the scope of the application, COGEM assumed that the application included viable material.

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