COMMISSIE
COGEM
GENETISCHE
MODIFICATIE

Aan de minister van
Volkshuisvesting, Ruimtelijke
Ordening en Milieubeheer
Mevrouw dr. J.M. Cramer
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DATUM 12 juni 2007
KENMERK CGM/070612-06
ONDERWERP Advice import of oilseed rape T45

Geachte mevrouw Cramer,

Naar aanleiding van een adviesvraag betreffende de import van genetisch gemodificeerd koolzaad van Bayer Cropscience AG., deelt de COGEM u het volgende mee.

Samenvatting:
De COGEM is gevraagd te adviseren over een vergunningaanvraag voor de import van genetisch gemodificeerd koolzaad T45. De koolzaadlijn bevat het *pat* gen waardoor de plant tolerant is voor glufosinaat-ammonium bevattende herbiciden.

Koolzaad T45 is niet meer commercieel interessant. Deze koolzaadvariant wordt niet meer geteeld en alle voorraden zijn vernietigd. Het kan echter niet volledig worden uitgesloten dat deze koolzaadvariant in kleine hoeveelheden gevonden kan worden bij import van ander koolzaad en dat het zich na een morsincident mogelijk enige tijd kan handhaven. Daarom heeft de aanvrager deze aanvraag ingediend.
De COGEM heeft eerder positief geadviseerd over genetisch gemodificeerd koolzaad dat een gen met soortgelijk werkingsmechanisme tot expressie bracht, coderend voor glufosinaat-ammonium tolerantie.

Er zijn wilde verwanten in Nederland waarmee koolzaad, in lage percentages, kan uitkruisen. Herbicidentolerante koolzaadplanten bezitten echter geen grotere verspreidingscapaciteit noch een hogere persistentie in het milieu dan niet-gemodificeerde planten. Het ingebrachte herbicidentolerantiegen geeft de plant alleen een selectief voordeel indien ze bespoten wordt met een glufosinaat-ammonium bevattend herbicide. Deze herbiciden worden alleen toegepast onder landbouwkundige omstandigheden en niet langs havenkades of wegbermen waar deze plant na een morsincident eventueel terecht kan komen.

De COGEM is van mening dat de risico's voor mens en milieu bij markttoelating voor import en verwerking van koolzaad T45 verwaarloosbaar klein zijn.

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

Hoogachtend,

Prof. dr. ir. Bastiaan C.J. Zoeteman
Voorzitter COGEM

c.c. Dr. ir. B.P. Loos
Dr. I. van der Leij

Import of genetically modified oilseed rape T45

COGEM advice CGM/070612-06

This notification concerns the import of genetically modified oilseed rape T45. T45 harbors the pat gene conferring tolerance to glufosinate-ammonium containing herbicides. Previously, COGEM advised positively on the import of oilseed rape variety Ms8 and Rf3 both expressing a gene which provides these plants with a similar mechanism for tolerance to these herbicides.

In the Netherlands, winter oilseed rape is being cultivated and several wild relatives are present outside agricultural environments. Under field conditions, oilseed rape is mainly a self-pollinating species, although out-crossing at low rates is possible with certain relatives. Oilseed rape T45 has no selective advantage except when a glufosinate-ammonium-containing herbicide is used. Glufosinate-ammonium is almost exclusively used in agricultural environments and not at roadsides, harbors, railways or near airports.

The event T45 is currently being phased out. Although oilseed rape T45 is not cultivated anymore and all stock has been destroyed, it cannot be ruled out that some residue might still be present in the field, resulting in adventitious presence of T45 when conventional oilseed rape is being cultivated. Environmental risks of T45 are therefore limited to spillage during transport of imported oilseed rape which might be contaminated with traces of T45. The T45 plants might possibly settle for some time after an eventual spillage incident.

In the opinion of COGEM, the applicant has sufficiently proven that no toxic or allergenic products are formed as a result of the genetically modified oilseed rape T45.

Based on these considerations, COGEM is of the opinion that the import of oilseed rape T45 poses a negligible risk to human health and the environment.

Introduction

The scope of the present notification by Bayer Cropscience AG concerns the import of oilseed rape variety T45. T45 expresses the *pat* gene, conferring tolerance to glufosinate-ammonium containing herbicides.

Previously, COGEM advised positively on the cultivation and import of oilseed rape varieties Ms8 and Rf3. Oilseed rape varieties Ms8 and Rf3 contain the *bar* gene, conferring tolerance to glufosinate-ammonium containing herbicides (8).

Aspects of the crop

Oilseed rape (*Brassica napus*) is a member of the family *Cruciferae* (*Brassicaceae*), together with wild cabbage (*B. oleracea*), turnip *(B. rapa)* and black mustard (*B. nigra*). *B. napus* (an amphidiploid / allotetraploid with chromosome n=19) originates from natural interspecific hybridization between the two diploid species *B. oleracea (n* = 9) and *B. rapa* (n = 10) (3). *B. napus* can be divided into the rutabaga, swede or (yellow) turnip and oilseed rape. Oilseed rape itself can be divided into a winter and a spring annual.

B. napus is grown as a winter annual in regions where winter conditions do not result in very low temperatures. The winter biotypes of oilseed rape typically require vernalisation before the onset of stem elongation, raceme development, flowering and seed set. In North America and northern parts of Europe, a spring biotype of *B. napus* is grown which requires no vernalisation prior to flowering. These biotypes have lower yields than the winter annual types, but require considerably less time to complete their life cycle. In the Netherlands spring oilseed rape is rarely cultivated because of the low yields (2).

Field mustard (*B. rapa L*) and black mustard (*B. nigra*) are part of the Dutch flora. For a long time, it was unclear whether oilseed rape had established itself in the Dutch flora or whether it was continuously volunteering from spilled seed (1). Nowadays, oilseed rape is considered part of the Dutch flora. Oilseed rape can be found as a cultivated crop and as a feral species in uncultivated terrain.

Under field conditions, oilseed rape is mainly a self-pollinating species, although out-crossing rates from 12 to 47 % between *B. napus* varieties have been reported (6). The heavy and sticky pollen from oilseed rape can be transferred from plant to plant through physical contact between neighboring plants and by wind and insects (3). Spreading of oilseed rape pollen decreases rapidly with increasing distance from the source of the pollen and windborne pollen makes only a negligible contribution to long-distance pollination of oilseed rape. Long distance spreading of pollen is presumably done by honeybees and bumblebees, which are attracted to the flowers because of the large quantities of nectar and pollen (4).

Seeds of the oilseed rape are small and produced in large quantities. Dependant on the oilseed rape cultivar, seeds of oilseed rape can persist in the soil for over four years under normal agricultural circumstances. If the soil remains uncultivated, even a dormancy period of 11 years has been observed (5).

Studies have pointed out that *B. napus* can outcross with several wild relatives. Pollination trials under field conditions indicate that oilseed rape can outcross with *B.*

rapa (rape seed), *B. juncea* (indian mustard), *Hirschfeldia incana* (shortpod mustard) and *Raphanus raphanistrum* (wild radish) (6,7). Gene flow can be influenced by many factors such as pollen viability, overlapping flowering period, distance between crop and weed and insect activity. The chromosome numbers and direction of crossing of the cultivated species and relatives are also important. Usually, oilseed rape hybrids are fertile but their pollen fertility and seed set are in most cases much poorer in the hybrids than in the pure species (6, 10, 11). Also, many hybrids fail to occur due to a mismatch in chromosome numbers (*napus*: 2n=38, *rapa* 2n=20, *nigra*=2n=16 en *oleracea*=2n=18). Generally, crosses between two species are possible if the female species has a polyploid level at least as high as the pollinating male species. For the development of hybrids, the ratio of maternal and paternal chromosomes must be at least of 2:1 or higher (9). The pollination of a tetraploid female parent by a diploid male usually produces seeds. The reciprocal cross, on the other hand, is sterile. Since *B. napus* is tetraploid, it will cross more easily with wild species (diploid) as a female parent, thus limiting gene flow. Moreover, hybridization between *B. napus* and wild relatives generally leads to a progeny with lower fitness, due to a mismatch in chromosome numbers (6, 10).

Molecular characterization

Oilseed rape T45 was produced by *Agrobacterium*-mediated transformation. An overview of the introduced sequences is given below:

Components of the insert in oilseed rape T45:

- LB, left border repeat derived from *Agrobacterium tumefaciens*
- P-35S, promoter derived from *Cauliflower mosaic virus* (CaMV)
- *Pat* gene, derived from *Streptomyces viridochromogenes* conferring tolerance to glufosinate-ammonium
- T-35S, terminating signal, derived from CaMV
- RB, Right border repeat, derived from *A. tumefaciens*

Properties of the introduced genes conferring herbicide tolerance

Oilseed rape T45 was genetically modified by the introduction of the *pat* gene, encoding the enzyme phosphinothricin acetyltransferase protein (PAT). Expression of PAT confers tolerance to glufosinate-ammonium herbicides.

The active ingredient in glufosinate-ammonium herbicide is L-phosphinothricin (L-PPT), which binds to glutamine synthetase in plants. The detoxification of excess ammonia is thereby prevented, leading to plant death. T45 expresses the *pat* gene which catalyses the conversion of L-PPT into an inactive form, which does not bind glutamine

synthetase. The application of glufosinate-ammonium herbicides to oilseed rape T45 will therefore be ineffective since ammonia is detoxified.

The PAT protein does not exhibit amino acid homology to known allergens or toxins.

Molecular analysis

The applicant performed an extensive bioinformatic analysis. The entire insert and flanking regions have been sequenced and analyzed. Southern blot analysis has proven that only one copy of the insert is present in the T45 genome and that no remains of the vector DNA are present.

COGEM is of the opinion that the molecular analysis of T45 was adequate and it is unlikely that toxic or allergenic products are formed as a consequence of the genetic modification. COGEM is of the opinion that the applicant has sufficiently proven that the PAT protein in T45 poses a negligible risk to human health and the environment.

Environmental Risk Assessment

Oil seed rape variety T45 has not been cultivated in Europe. The event T45 is currently being phased out. Oilseed rape T45 is not commercially participating on the market anymore. From 2003 on Bayer started to withdraw T45 from the market and T45 seeds are no longer sold from the beginning of 2006. Bayer and all third parties with licenses for the production and sale of T45 certified the destruction of all existing stock. Although oilseed rape is not cultivated anymore and all stock has been destroyed, it cannot be ruled out that some residue is still present in the field, for instance in the soil- seed-bank or in the processing chain. This might lead to inadvertent presence of T45 when conventional oilseed rape is cultivated. When this oilseed rape is exported, a contamination with T45 might be present.

Environmental risks identified in relation to the import of oilseed rape concern spillage of oilseed rape during transport or at the location of turnover points. Spilled oilseed rape might contain traces of T45 oilseed rape which can grow and establish itself at that location. Also, this oilseed rape might be able to outcross with wild relatives and wildtype *B. napus* in the area. However, T45 or its progeny has no selective advantage compared to conventional oilseed rape unless glufosinate-ammonium is used. In general, glufosinate-ammonium containing herbicides are not used outside an agricultural environment, because it is expensive and less effective than other broad spectrum herbicides, like glyfosate. Also, the last traces of T45 oilseed rape will disappear over time, since seed production and cultivation of T45 has stopped. Outcrossing of oilseed rape might occur at very low levels producing hybrids which are in most cases less-fertile than the non-hybrid species (6,10).

Advice

The present application concerns the import of oilseed rape variety T45. T45 expresses the *pat* gene, conferring tolerance to glufosinate-ammonium containing herbicides.

In the opinion of COGEM, the applicant has sufficiently proven that no toxic or allergenic products are formed as a result of the genetically modified oilseed rape T45.

In the Netherlands, winter oilseed rape is being cultivated and several wild relatives are present outside agricultural environments. Under field conditions, oilseed rape is mainly a self-pollinating species, although out-crossing at low rates is possible with certain relatives.

Furthermore, COGEM notices that spillage of oilseed rape can lead to volunteers. The applicant stated that operators handling transport are trained to avoid or minimize spillage and to ensure that spills are cleaned up. In the opinion of COGEM, spillage of genetically modified oilseed rape T45 does not pose an environmental risk because T45 has no selective advantage, except when glufosinate-ammonium is applied. In general, glufosinate-ammonium containing herbicides are not used outside an agricultural environment. However, spillage and volunteers of GM-oilseed rape might lead to societal discussion, which requires adequate communication on the assessed absence of environmental risks by the companies and authorities involved.

Based on the above mentioned considerations, COGEM is of the opinion that the proposed import of oilseed rape variety T45 poses a negligible risk to human health and the environment.

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