COMMISSIE
COGEM
GENETISCHE
MODIFICATIE

Aan de minister van
Volkshuisvesting, Ruimtelijke
Ordening en Milieubeheer
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Datum 19 april 2007
Kenmerk CGM/070419-02
Onderwerp Advies Import of genetically modified maize line MON88017 x MON810

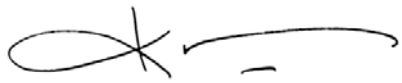
Geachte mevrouw Cramer,

Naar aanleiding van een adviesvraag betreffende de import en verwerking van genetisch gemodificeerde maïs MON88017 x MON810 van Monsanto Europe S.A., deelt de COGEM u het volgende mee.

Samenvatting:
De COGEM is gevraagd te adviseren over de mogelijke risico's voor mens en milieu van import en verwerking van de genetisch gemodificeerde (gg) maïslijn MON88017 x MON810. Teelt van deze lijn maakt geen deel uit van de vergunningaanvraag.
In de maïslijn is het gen cp4*epsps* ingebouwd waardoor de plant tolerant is voor glyfosaat bevattende herbiciden. Daarnaast bevat de maïslijn de genen *cry3Bb1* en *cry1Ab* waardoor de plant resistent is voor bepaalde insecten uit de orde van de *Coleoptera*, zoals de maïswortelkever en de *Lepidoptera*, zoals de Europese maïsboorder.
Maïs heeft in Nederland geen wilde verwanten en opslag van maïsplanten is in Nederland nagenoeg uitgesloten. Verwildering van de maïsplant is in Nederland nooit waargenomen. Er zijn geen redenen om aan te nemen dat de modificaties het verwilderingspotentieel vergroten. De COGEM acht daarom de kans verwaarloosbaar klein dat incidenteel morsen leidt tot verspreiding van MON88017 x MON810 binnen Nederland.
De COGEM is van mening dat de moleculaire karakterisering van de maïslijn MON88017 x MON810 volledig is en dat de aanvrager voldoende heeft aangetoond dat er geen nieuwe allergene of toxische producten gevormd worden als gevolg van de kruising van de lijnen .
Derhalve acht de COGEM de risico's voor mens en milieu verwaarloosbaar klein bij markttoelating voor import en verwerking van de maïslijn MON88017 x MON810.

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

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Import of genetically modified maize line MON88017 x MON810

COGEM advice CGM/070419-02

This notification concerns the import and processing of the genetically modified maize line MON88017 x MON810 for use in feed and food. The maize line harbors the genes cp4epsps, cry1Ab and cry3Bb1. Cp4epsps confers tolerance to herbicides containing the active ingredient glyphosate. The cry3Bb1 and Cry1Ab genes are responsible for resistance to certain coleopteran and lepidopteran insects such as the corn rootworm (Diabrotica virgifera) and the European corn borer (Ostrinia nubilalis), respectively. Cultivation is not part of the application.

Previously, COGEM advised positively on the import of maize line MON88017 which expresses the cp4epsps gene and the cry3Bb1 gene. In 1996, COGEM has given a positive advice on the cultivation of maize line MON810 which expresses the cry1Ab gene.

In the Netherlands, no wild relatives of maize are present and establishment of maize plants in the wild has never been observed. There are no reasons to assume that the inserted traits will increase the potential of the maize line to establish feral populations. In addition, the appearance of volunteers is very rare under Dutch conditions.

COGEM is of the opinion that interactions between recombinant gene products are not expected. The applicant has sufficiently proven that no toxic or allergenic products are formed as a result of the modification of maize line MON88017 x MON810. Based on these considerations, COGEM is of the opinion that the import of maize line MON88017x MON810 poses a negligible risk to human health and the environment.

1. Introduction

The scope of the present notification by Monsanto Company concerns the import and processing of maize line MON88017 x MON810 for use in feed and food.

The maize line is produced by traditional crossing of maize lines MON88017 and MON810. Maize line MON88017 expresses the genes *cp4epsps* and *cry3Bb1* conferring tolerance to glyphosate containing herbicides and insect resistance to certain coleopteran insects such as the corn rootworm *(Diabrotica virgifera)*. Maize line MON810 expresses Cry1Ab conferring resistance to certain lepidopteran insects such as the European corn borer.

1.1 Previous COGEM advices

Recently, COGEM advised positively on the import and processing of parental maize line MON88017, which expresses the genes *cp4epsps* and *cry3Bb1* (11).

In 1996 COGEM has also advised positively on the cultivation of the other parental maize line MON810, containing the gene *cry1Ab* (10). Additionally, maize line MON810 is already commercially grown in the U.S.A.

2. Maize line MON88017 x MON810

2.1 Aspects of the crop

Maize (*Zea mays L.*) is a member of the grass family *Poaceae.* Maize is being cultivated as an agricultural crop, originating from Central America. Although insect pollination can not be completely excluded, maize is predominantly wind pollinated (1;2). According to literature, pollen viability varies between 30 minutes and 9 days (2;3;4). In Europe, no wild relatives of maize are present and, therefore, hybridization with other species can not occur.

The appearance of volunteers is very rare under Dutch conditions. Grains exhibit no germination dormacy, resulting in a short persistence under Northern European climate conditions. In addition, only few seeds remain on the field after harvesting of fodder maize (1). Establishment of maize plants in the wild has never been observed in the Netherlands.

2.2 Molecular characterization

The genetically modified maize line MON88017 x MON810 was produced by crossing two parental maize lines MON88017 and MON810.
An overview of the introduced sequences is given below:

Summary of the genetic elements inserted in MON88017:

- Cry3Bb1 expression cassette:
  - P-e35S, originating from *Cauliflower mosaic virus* (CaMV), promoter with a duplicated enhancer region
  - Wt CAB leader, originated from *Triticum aestivum* (wheat); 5' untranslated leader of the wheat chlorophyll a/b binding protein
  - Ract1 intron, derived from *Oryza sativa* (rice); promotes transcription
  - MON88017 *cry3Bb1*, originating from *Bacillus thuringiensis subsp. kumamotoensis*, DNA sequence coding for a genetic variant of CryBb1 protein.
  - Tahsp 17 3', wheat heat shock protein derived from *T. aestivum*; stops transcription and induces the polyadenylation

- Cp4epsps expression cassette:

- P-ract1/ ract1 intron, promoter and intron derived from *O. sativa*, intron promotes transcription
- *Ctp2* gene from *Arabidopsis thaliana*; encoding the N-terminal chloroplast transit peptide
- *Cp4epsps* gene from *Agrobacterium. tumefaciens* CP4; encoding 5-enolpyruvlshikimate-3-phosphatesynthase (epsps)
- NOS 3', terminator from *A. tumefaciens*; terminates transcription

Summary of the elements inserted in MON810:

- Cry1Ab gene cassette:
  - P-e35S, originating from the CaMv, containing a portion of the CaMv promoter with the duplicated enhancer region and 5' untranslated region
  - Zmhsp70 intron, DNA sequence derived from maize containing the intron sequence from the maize *hsp70* gene (heat shock protein) present to stabilize the level of gene transcription
  - Cry1Ab, DNA sequence containing synthetic linker and a portion of the synthetic coding sequence for a variant of the Cry1Ab protein from *B. thuringiensis subsp. Kurstaki.*

Properties of the introduced genes conferring herbicide tolerance

Cp4epsps encodes Cp4epsps proteins possessing a high tolerance to glyphosate. With the introduction of *cp4epsps* from *A. tumefaciens* in maize MON88017 tolerance was obtained to glyphosate containing herbicides.

Epsps is a natural occurring enzyme involved in the biosynthesis of aromatic amino acids. In non-transgenic maize lines, glyphosate acts by binding to, and thus inhibiting the function of naturally occurring epsps. Consequently, aromatic amino acids are no longer formed, leading to plant death. In contrast, Cp4epsps is not affected by glyphosate because of a reduced binding affinity. MON88017 expresses the gene *cp4epsps*, resulting in a high tolerance to glyphosate. The application of this herbicide will therefore not cause death of maize line MON88017, because the plant is still able to produce aromatic amino acids (5).

Epsps proteins are active in the chloroplasts of a plant cell. The *ctp2* gene is fused to the epsps transgene, resulting in the transport of the transgenic Epsps protein to the chloroplast (7).

Properties of the introduced genes conferring insect resistence

MON88017 x MON810 was genetically modified with the *cry3Bb1* gene derived from *B. thuringiensis* (subsp. *kumamotoensis*). The produced Cry3Bb1, a δ-endotoxin, is lethal to

certain insects of the coleopteran order, including larvae of the corn rootworm (*Diabrotica sp.).* The δ-endotoxin selectively binds to receptors located in the midgut of susceptible insects (6). After this binding to receptors, the gut is perforated, enabling enterobacteria from the midgut to enter the body, causing the insect to die from poisoning within 48 to 120 hours (9).

The corn rootworm is an economically important pest insect, which causes major crop losses. Larvae of this insect feed on maize roots, resulting in the interference of the plant's ability to absorb water and nutrients and in the reduction of the stability of the plant. As a consequence, damaged plants may lodge, making harvesting difficult.

Corn rootworm was accidentally introduced in the mid-nineties in Bosnia, presumably by military air traffic, and became established shortly after introduction. The pest is still spreading at regular rate of about 40 km per year, but is infamous for rapid spread over large distances by (air) traffic. In 2003 this insect was first discovered near the airport of Schiphol in the Netherlands, but eradicated successfully. If the corn rootworm is able to establish and spread itself in the Netherlands, large crop damage can be expected (8).

MON88017 x MON810 also expresses the Cry1Ab gene derived from *B. thuringiensis* (subsp. *Kurstaki*). Cry1Ab encodes another δ-endotoxin, which is lethal to insects of the lepidopteran order, including larvae of the European corn borer *(Ostrinia nubilalis)* and the pink borer *(Sesamia cretica).* Cry1Ab has the same physical effect on the insects as Cry3Bb1.

The larvae of the European corn borer cause severe damage to corn crops by feeding on the stalks and creating boreholes. This results in weakened plants, eventually causing the plant to fall over. The damaged plants are also more susceptible to molds and rot. Furthermore, larvae can feed on the kernel causing a reduction of grain quality. The European corn borer is a pest insect in the United States and Canada. In the Netherlands however, this insect species is not of agronomic interest because the crop consists mainly of fodder maize. Together with the fodder maize, the pupae of the corn borer are chopped during harvesting; therefore, the corn borer population is not able to establish itself. In addition, the climate in the Netherlands is not optimal for the European corn borer.

Molecular analysis

Previously, the molecular aspects of parental maize line MON88017 as well as MON810 have been thoroughly analyzed. COGEM is of the opinion that the molecular analysis of both parental maize lines MON88017 and MON810 was sufficient and it is unlikely that toxic or allergenic products are formed as a consequence of the modifications. Therefore, COGEM is of the opinion that by traditional crossing of these two maize lines, no adverse effects are to be expected as a consequence of the modifications.

The proteins encoded by the genes *cp4epsps*, *cry3Bb1* and *cry1Ab* do not exhibit amino acid homology or protein structure homology to known allergens or toxins. In addition, the expression levels of *cp4epsps*, *cry3Bb1* and *cry1Ab* in the hybrid maize line MON88017 x MON810 are similar to the levels of expression in the parental maize lines. To the opinion of the COGEM, interactions between recombinant gene products are not expected in the hybrid maize line MON88017 x MON810 because the gene products accumulate in different cellular compartments. The proteins Cry3Bb1 and Cry1Ab accumulate in the cytoplasm, while Cp4epsps is directed to the chloroplasts.
Based on the data concerning feeding study experiments with MON88017 x MON810 provided by the applicant, COGEM agrees that no adverse effects are likely to occur in case of incidental consumption of MON88017 x MON810 or products that contain MON88017 x MON810.

Furthermore, the genes and their gene products have a history of safe use. Products and derivatives of the maize lines expressing these genes have been handled and consumed without reports of side-effects.

3. Advice

The present application concerns the commercial import and processing of maize line MON88017 x MON810 for the use in food and feed. MON88017 x MON810 expresses the genes *cp4epsps*, *cry3Bb1 and cry1Ab*, providing the plant with a herbicide tolerance trait as well as resistance to certain coleopteran and lepidopteran insects, respectively. In the past, COGEM advised positively on maize lines with these traits and hybrid maize lines with a combination of these traits.

There are no wild relatives of maize in the Netherlands and the appearance of volunteers is very rare under Dutch conditions. Furthermore, there are no reasons to assume that the inserted traits will increase the now absent potential of the maize line to run wild.
In the opinion of COGEM, the molecular analysis is adequate. The inserted genes and gene products possess a history of safe use and no interactions between recombinant gene products are expected in the modified maize line MON88017 x MON810. Moreover, based on the data from feeding study experiments with MON88017 x MON810, no adverse effects are to be expected in case of occasional consumption of MON88017 x MON810 or products that contain MON88017 x MON810. In addition, maize line MON810 is already commercially grown and consumed in the U.S.A without any reports of adverse effects. Therefore, COGEM is of the opinion that no toxic or allergenic products are formed as a consequence of the crossing of maize lines MON88017 x MON810.

In view of these considerations, COGEM is of the opinion that the proposed import and processing of maize line MON88017 x MON810 poses a negligible risk to human health and the environment.

Additional remarks

Maize is not able to run wild under Dutch climate conditions. However, in case of extreme climate changes due to global warming, it can not be excluded that conditions for survival of maize in the Netherlands might change. It is unlikely but theoretically possible that climate changes turn out in favor of maize on the long term. Additionally, climate changes will probably first of all cause effect in the southern parts of Europe. COGEM will follow these developments closely.

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