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Uw kenmerk	Uw brief van	Kenmerk	Datum
EFSA/GMO/BE/2004/07	27 januari 2005	CGM/050228-02	28 februari 2005

Onderwerp
Advies Marktdossier EFSA/GMO/BE/2004/07
Insectenresistente en herbicidentolerante maïs MON863 x MON810 x NK603

Geachte heer Van Geel,

Naar aanleiding van het dossier EFSA/GMO/BE/2004/07, 'MON863 x MON810 x NK603' betreffende de import en verwerking van genetisch gemodificeerde maïs door Monsanto Europe S.A., adviseert de COGEM als volgt.

Samenvatting:
De COGEM is gevraagd te adviseren over de import en verwerking van een hybride maïslijn (kruising tussen de genetisch gemodificeerde ouderlijnen MON863, MON810 en NK603). De teelt van deze lijn maakt geen deel uit van de vergunningaanvraag.
De ouderlijnen bezitten de genen c*ry3Bb1*, c*ry1A(b)* en *cp4 epsps* waardoor ze minder gevoelig zijn voor bepaalde kever- en vlinderachtige insecten en tolerant zijn voor herbiciden met als werkzame stof glyfosaat.

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 is geen reden om aan te nemen dat modificatie het verwilderings-potentieel vergroot. De COGEM is daarom van mening dat het incidenteel morsen van de genetisch gemodificeerde maïs in Nederland geen risico's voor het milieu met zich meebrengt. De ingebrachte genen en de genproducten kennen een geschiedenis van veilig gebruik en interacties tussen de genproducten worden niet verwacht. Er is tevens voldoende bewijs geleverd dat er geen nieuwe allergene of toxische producten gevormd worden als gevolg van de kruising. De COGEM acht derhalve de risico's voor mens en milieu bij de import en verwerking van onderhavige maïslijn verwaarloosbaar klein.

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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. Dr. ir. B.P. Loos
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Title: Import and processing of insect resistant and herbicide tolerant maize MON863 x MON810 x NK603 (EFSA/GMO/BE/2004/07)

COGEM advice: CGM/050228-02

The present application concerns the commercial import and processing for use in feed and food of a hybrid genetically modified maize line. Cultivation is not part of the application. The hybrid maize line is developed through traditional breeding of the genetically modified maize lines MON863, MON810 and NK603. These parental lines contain genes (cry3Bb1, cry1A(b) and cp4 epsps) conferring resistance to certain coleopteran and lepidopteran insects, and tolerance to herbicides containing the active ingredient glyphosate.

In the Netherlands, no wild relatives of maize are present and the appearance of volunteers is never a problem under Dutch conditions. Establishment of maize plants in the wild has never been observed. There are no reasons to assume that the inserted traits will increase the now absent potential of the maize line to establish feral populations. Therefore, COGEM is of the opinion that incidental spillage of the hybrid maize line will pose no risk to the environment in the Netherlands.

The inserted genes and their gene products have a history of safe use in maize lines and interactions between recombinant gene products are not expected. Furthermore, it is sufficiently proven that no toxic or allergenic products are formed as a result of the crossing of the three genetically modified maize lines.

In view of the aforementioned, COGEM is of the opinion that the risks for the environment and human health associated with the import and processing of the hybrid maize line MON863 x MON810 x NK603 are negligible.

Introduction

The present application concerns the commercial import and processing for use in feed and food of a hybrid maize line.

The hybrid maize line MON863 x MON810 x NK603 is developed through traditional breeding methods by the crossing of the genetically modified maize lines MON863, MON810 and NK603. Thereby, MON863 x MON810 x NK603 inherits and effectively combines the three single-traits of the parental maize lines. The MON863 line is modified by the introduction and expression of the gene c*ry3Bb1* that confers tolerance to certain coleopteran insects, especially the larvae of the corn rootworm (*Diabrotica* spp.). By insertion and expression of the gene c*ry1A(b)*, MON810 has obtained resistance to certain insects of the lepidopteran order, including the European corn borer (*Ostrinia nubilalis*) and 'pink borer' (*Sesamia*

spp.). The Maize line NK603 contains and expresses the genes cp*4 epsps* and cp*4 epsps L214P*. These genes provide tolerance to herbicides containing the active ingredient glyphosate.

The maize lines MON810 and NK603 have been previously approved for commercial import and processing in the E.U. (C/FR/95/12-02 and C/ES/00/01). MON863 is currently waiting for approval for import and processing in the E.U. (C/DE/02/09). Moreover, maize lines MON863 x MON810, MON863 x NK603 and NK603 x MON810 are already commercially grown in the U.S.A.. There are no reports of side-effects concerning handling and consuming products and derivatives of this line.

Previous COGEM advices

In the past COGEM has advised positively on the genetically modified parental maize lines MON810, NK603 and MON863 for import and processing (CGM/960807-01, CGM/030319-08 and CGM/031016-04).

Furthermore, in 2003 and 2004 the stacked-trait maize lines MON863 x MON810 and NK603 x MON810 were evaluated positively (CGM/031016-04 and CGM/040421-01).

Aspects of the crop

Maize (*Zea mays* L.) is a member of the grass family *Poaceae* and cultivation of maize, as an agricultural crop, originated in Central America. Maize is predominantly wind pollinated, although insect pollination can not be completely excluded (1;2). Pollen viability varies between 30 minutes and 9 days according to literature (2;3;4). In Europe, no wild relatives of maize are present and, therefore, hybridisation with other species will not occur.

The appearance of volunteers is very rare under Dutch conditions. Maize kernels exhibit no germination dormancy, resulting in a short persistence. Furthermore, during harvesting of fodder maize only few seeds remain on the field (1). Establishment of maize plants in the wild has never been observed in the Netherlands. There are no reasons to assume that inserted traits will increase the potential of the maize line to run wild.

Molecular characterisation

Origin and function of the introduced genes

The present stacked-trait maize line is established by crossing three genetically modified single-trait maize lines. The characterization of the parental lines will be discussed below.

Origin and function of the introduced genes in MON863

Maize line MON863 is genetically modified by means of particle bombardment. The introduced gene c*ry3Bb1* confers resistance to coleopteran insects like the corn rootworm. By inserting the *nptII* gene, the plant acquires tolerance to kanamycin.

An overview of the introduced sequences is given below:

• c*ry3Bb1* expression cassette

- 4AS1, derived from the *Cauliflower mosaic virus* (CaMV); promoter associated with high protein expression levels in the root
- *wt CAB*, originated from *Triticum aestivum* (wheat); promotes translation
- ract1 intron, derived from *Oryza sativa* (rice); promotes transcription
- *cry3Bb1*, from *Baccilus thuringiensis* subsp. *kumamotoensis*; confers resistance to coleopteran insects
- tahsp 17 3', derived from *T. aestivum*; stops transcription and directs polyadenylation

• *nptII* gene cassette:

- 35S promotor, derived from the CaMV; constitutive promoter
- *nptII* gene of *Escherichia coli* Tn5; encoding neomycin phosphotransferase (kanamycinresistance)
- *ble* (truncated), originated from *E. coli* Tn5; encoding a non-functional bleomycine resistance
- Nos 3' terminator from *Agrobacterium tumefaciens*

Origin and function of the introduced genes in MON810

Maize line MON810 is genetically modified by means of 'particle bombardment'. The introduced c*ry1A(b)* gene confers resistance to certain insects of the order *Lepidoptera* and particularly to the larvae of the European corn borer (*Ostrinia nubilalis)*.

The vector contains the following elements:

- E35S, derived from the CaMV; constitutive promoter
- *hsp7*, intron derived from *Zea mays*. *Hsp7*; stabilises gene transcription.
- c*ryIA(b)*, gene derived from *Bacillus thuringiensis* subsp. *Kurstaki*; encoding for the c*ry1A(b)* δ-endotoxin.

Origin and function of the introduced genes in NK603

The genetically modified maize line NK603 was also produced by particle bombardment. A restriction fragment of plasmid PV-ZMGT32, containing both *cp4 epsps* expression cassettes was inserted into the plant. The EPSPS proteins confer tolerance to herbicides containing glyphosate.

The two expression cassettes contain the following sequences:

• *cp4 epsps* expression cassette 1:

- P-ract1/ract1 intron, promotor and intron derived from *O. sativa*; intron promotes transcription
- *ctp2* gene from *Arabidopsis thaliana*; encoding a chloroplast transit peptide
- *cp4 epsps* gene derived from *A. tumefaciens CP4*; encoding 5-enolpyruvylshikimate-3-phophatesynthase (EPSPS)
- Nos 3', terminator from *A. tumefaciens*; stops transcription

• *cp4 epsps* expression cassette 2:

- E35S, constitutive promotor from CaMV
- *hsp70*, intron derived from *Z. mays*; stabilises transcription
- *ctp2 gene* derived from *A. thaliana*; encoding a chloroplast transit peptide
- *cp4 epsps L214P*, gene derived from *A. tumefaciens CP4*, encoding EPSPS
- Nos 3', terminator from *A. tumefaciens*

Properties of the introduced genes

Insect resistance

Insect resistance of maize is increased by genetically modifying the plants. By inserting the genes c*ry3Bb1* and c*ry1A(b)*, plants will start to produce δ-endotoxins (Bt-toxins). These toxins act as insecticide only when eaten by larvae. Following eating, the toxins selectively bind to receptors located in the midgut, resulting in gut perforation causing death of the insect within 48 to 72 hours (5). Both genes will be discussed below.

cry3Bb1

The c*ry3Bb1* gene is derived from *B. thuringiensis* (subsp. *Kumamotoensis*) and inserted in maize line MON863. The produced toxin is lethal to insects of the *Coleoptera* order, including larvae of the corn rootworm (*Diabrotica* sp).

The corn rootworm is an economically important pest insect, causing major crop losses. Larvae of this insect feed on 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.

The corn rootworm was accidentally introduced in the mid-nineties in Bosnia by military air traffic and became established shortly after introduction. The pest is still spreading at a 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 Schiphol airport in the Netherlands, but eradicated successfully. When the corn rootworm is able to establish and spread itself in the Netherlands, large damage to crops can be expected (6;7). Successful introduction by (air) traffic might occur anytime in the near future.

The Cry3Bb1 protein does not exhibit amino acid homology or protein structure homology to known allergens or toxins.

cry1A(b)

The cry1A(b) gene is derived from *B. thuringiensis* (subsp. *Kurstaki*) and introduced in line MON810. The gene encodes for a protein which is toxic to insects of the *Lepidoptera* order, especially to the larvae of the European corn borer (*Ostrinia nubilalis*) and the 'pink borer' (*Seasmia cretica*).

The larvae damage maize by boring the stalks and causing tunneling. This results in weakened plants and allows entrance of molds and rots. 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, 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.

The Cry1A(b) protein does not exhibit amino acid homology or protein structure homology to known allergens or toxins.

Herbicide tolerance

Maize line NK603 was genetically modified with 2 *cp4 epsps* genes encoding EPSPS. In this way tolerance was obtained to glyphosate herbicides.

Glyphosate inhibits the function of EPSPS, an enzyme involved in the biosynthesis of aromatic amino acids. By binding of glyphosate to EPSPS, aromatic aminoacids are no longer formed, leading to plant death. Maize line NK603 expresses *cp4 epsps* genes which possess a naturally high tolerance to glyphosate. The application of glyphosate will therefore not cause death of maize line NK 603 of MON863 x NK603, because the plant is still able to produce aromatic amino acids.

EPSPS proteins are active in the chloroplasts of a plant cell. The *cpt2* gene is fused to the *epsps* gene, resulting in the transport of the transgenic EPSPS protein to the chloroplast (8).

Molecular analysis

The applicant provided information, based on southern blot analysis, that the integration patterns of the introduced genes in the parental lines remain stable en unchanged in the upcoming generations.

To the opinion of COGEM, interactions between recombinant gene products are not expected in the hybrid maize line, because the gene products accumulate in different cellular compartments. The proteins Cry3Bb1, Cry1A(b) and NPTII

accumulate in the cytoplasm, while CP4 EPSPS is directed to the chloroplasts. Furthermore, the inserted genes and their gene products have a history of safe use through handling and consumption of maize lines. The parental lines have also been crossed, resulting in three double-trait products (MON863 x MON810, MON863 x NK603 and NK603 x MON810), and are already commercially available in the U.S.A.. Products and derivatives of the lines have been handled and consumed without reports of side-effects.

It is sufficiently proven that no toxic or allergenic products are formed as a result of the crossing.

Advice

The present application concerns the import, processing and feed and food use of a hybrid maize line. Cultivation of the present line is not part of the application.

There are no reasons to assume that the inserted traits will increase the now absent potential of the maize line to establish feral populations. Therefore, COGEM is of the opinion that incidental spillage of maize kernels MON863 x MON810 x NK603 will pose no risk in the Netherlands.

The inserted genes and their gene products have a history of safe use and no interactions between recombinant gene products are therefore expected in the hybrid maize line. Furthermore, in the opinion of COGEM, it is sufficiently proven that no toxic or allergenic products are formed as a result of the crossing.

In view of the above-mentioned, COGEM is of the opinion that the risks for the environment and human health associated with the import and processing of the hybrid maize line MON863 x MON810 x NK603 are negligible.

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