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Uw kenmerk	Uw brief van	Kenmerk	Datum
EFSA/GMO/UK/2004/05	28 april 2005	CGM/050526-01	26 mei 2005

Onderwerp
Advies Marktdossier EFSA/GMO/UK/2004/05
Insectenresistente en herbicidentolerante maïs 1507 x NK603

Geachte heer Van Geel,

Naar aanleiding van het dossier EFSA/GMO/UK/2004/05, '1507 x NK603' voor de import en verwerking van genetisch gemodificeerde maïs door Pioneer Hi-Bred International Inc. adviseert de COGEM als volgt.

Samenvatting:

De COGEM is gevraagd te adviseren over de mogelijke risico's voor mens en milieu betreffende import en verwerking van een genetisch gemodificeerde (gg-) maïslijn (kruising tussen de gg-lijn 1507 en NK603). Teelt van deze maïslijn maakt geen deel uit van de vergunningaanvraag. Door insertie van het *cry1F* gen en het *pat* gen in de ouderlijn 1507 is respectievelijk resistentie voor vlinders en motten, waaronder de Europese maïsboorder, en tolerantie voor herbiciden met als werkzame stof glufosinaat-ammonium, verkregen. Hiernaast is de maïslijn door insertie van het *cp4 epsps* gen in NK603, tolerant geworden voor glyfosaat bevattende herbiciden.

Maïs kent zoals in eerdere adviezen vermeld, geen wilde verwanten in Nederland en opslag van maïsplanten is hier niet van landbouwkundige betekenis. Verwildering van de maïsplant in Nederland is nog nooit waargenomen. Er zijn geen redenen om aan te nemen dat de modificatie het verwilderingspotentieel vergroot. Hiernaast worden interacties tussen de genproducten niet verwacht. Tevens is er voldoende bewijs geleverd dat er geen nieuwe allergene of toxische producten gevormd worden als gevolg van de kruising tussen de twee ouderlijnen.

De COGEM acht derhalve de risico's voor mens en milieu bij de import en verwerking van

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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 1507 x NK603

COGEM advice: CGM/0505xx-xx

The present application concerns the commercial import and processing for use in feed and food of a genetically modified hybrid maize line. Cultivation is not part of the application.

*The hybrid maize line is developed through traditional breeding of the genetically modified maize lines 1507 and NK603. These parental lines contain genes (cry*1F*, pat and cp4 epsps) conferring resistance to certain lepidopteran insects, and tolerance to herbicides containing the active ingredient glufosinate-ammonium or glyphosate.*

Maize line 1507 x NK603 is already commercially grown in the U.S.A.

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. Therefore, COGEM is of the opinion that incidental spillage of the hybrid maize line will pose no risk to the environment in the Netherlands.

Interactions between recombinant gene products are not expected. It is sufficiently proven that no toxic or allergenic products are formed as a result of the crossing of the two genetically modified maize lines.

Based on these considerations, COGEM is of the opinion that the proposed import and processing of the maize line 1507 x NK603 does pose a negligible risk to human health and the environment.

Introduction

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

The hybrid maize line 1507 x NK603 is developed through traditional breeding methods by crossing the genetically modified maize lines 1507 and NK603. Thereby, 1507 x NK603 inherits and effectively combines the three single-traits of the parental maize lines. The 1507 line is modified by the introduction and expression of the gene *cry*1F that confers resistance to certain lepidopteran insect pests, like the larvae of the European corn borer (*Ostrinia nubilalis*) and *Sesamia* spp. like the pink borer. Besides, the inserted *pat* gene is conferring tolerance to glufosinate-ammonium based herbicides. The maize line NK603 contains and expresses the genes *cp4 epsps* and *cp4 epsps L214P*. These genes confer tolerance to herbicides containing the active ingredient glyphosate.

The maize line NK603 has been previously approved for commercial import and processing in the EU (C/ES/00/01). Maize line 1507 is waiting for approval by the European environmental counsel.
Maize line 1507 x NK603 is already commercially grown in the U.S.A. There are no reports of adverse health 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 NK603 and 1507 for import and processing (CGM/030319-08, CGM/030115-01 and CGM/030919-04).

Furthermore, in 2004 and 2005 the stacked-trait maize lines NK603 x MON810, NK603 x MON863 and NK603 x MON863 x MON810 were evaluated positively (CGM/040421-01, CGM/050228-03 and CGM/050228-02).

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 (1,3). According to literature, pollen viability varies between 30 minutes and 9 days (2,3,4). There are no wild relatives of maize in Europe and, therefore, it is not possible that maize will hybridise with other species.

The appearance of volunteers is very rare under Dutch conditions. Grains do not possess dormancy, resulting in a short persistence. Furthermore, after harvesting of fodder maize only few seeds remain on the field (1). In the Netherlands, maize has never established itself in the wild. There are no reasons to assume that inserted traits will increase the potential of the maize line to establish feral populations.

Molecular characterisation
The present stacked-trait maize line is established by crossing of two genetically modified single-trait maize lines (1507 and NK603). The characterization of these parental lines will be discussed below.

Origin and function of the introduced genes in 1507
Maize line 1507 is genetically modified by means of particle bombardment. A gene fragment containing the *cry*1F and the *pat* gene was inserted into the maize variety. The *cry*1F gene confers resistance to lepidopteran insects like the European maize borer. By inserting the pat gene, the plant acquires tolerance for herbicides with the active ingredient glufosinate-ammonium.

An overview of the introduced sequences is given below:

• *cry*1F expression cassette

- *ubi*MZ1(2), ubiquitine promotor (plus 5'untranslated region) derived from the *Zea mays*
- *cry*1F, synthetic version of truncated *cry*1F gene from *Baccilus thuringiensis* subsp. *aizawai*;
- ORF25PolyA, terminator derived from *Agrobacterium tumefaciens* pTi 15955; stops transcription and induces the polyadenylation

• *pat* gene cassette:

- 35S promotor, derived from the cauliflower mosaic virus (CaMV); constitutive promoter
- *pat* gene of *Streptomyces viridochromogenens* strain Tü494; encoding phosphinothricin-N-acteyltransferase (PAT)
- terminator, from CaMV

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-ZMGT32L, containing both *cp4 epsps* expression cassettes was inserted into the plant. The EPSPS protein confers tolerance to the herbicides containing the active ingredient 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 conferring insect resistance

Maize line 1507 was genetically modified with the *cry*1F gene derived from *B. thuringiensis* (sbsp. *aizawai*). The produced Cry1F, a δ-endotoxin, is lethal to insects

of the *Lepidoptera* order, including larvae of the European maize borer (*Ostrinia nubilalis*) and of the *Sesamia* spp like the pink borer. The δ-endotoxin selectively binds to receptors located in the midgut of susceptible insects. Following binding, the gut is perforated causing death of the insect. Cry1F is only lethal when eaten by the larvae of certain lepidopteran insects. Its specificity of action is directly related to the presence of certain binding sites in the target insects. Mammalian intestinal cells bare such binding sites. Therefore, humans and insects are not susceptible to these proteins.

The Cry1F protein does not exhibit amino acid homology or protein structure homology to known allergens or toxins.

Properties of the introduced genes conferring herbicide tolerance

The *pat* gene encoding phosphinothricin-N-acteyltransferase, is present in maize line 1507. The *pat* gene confers tolerance to glufosinate-ammonium based herbicides.

Glufosinate inhibits an enzyme, called glutamine synthetase, which is involved in the synthesis of the amino acid glutamine (6). Glutamine synthetase is also involved in ammonia detoxification. By inhibiting glutamine synthase, glutamine levels in the plant are reducing and the concentrations of ammonia in plant tissues are increasing. This leads to cell membrane disruption and termination of photosynthesis, resulting in plant withering and death.

By expressing the *pat* gene, the acetylation for glufosinate is catalysed resulting in detoxification of glufosinate into an inactive compound (7).

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 NK603 or 1507 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 sequence encoding the chloroplast transit peptide is fused to the *epsps* gene, resulting in the transport of the transgenic EPSPS protein to the chloroplast (5).

Molecular analysis

To the opinion of the experts of COGEM, interactions between recombinant gene products are not expected in the hybrid maize line. The enzymatic reaction of the (bacterial) EPSPS gene product is the same as the natural occurring vegetable EPSPS (bacterial EPSPS differs in the sensitivity for glyphosate). Therefore, maize line 1507 combines already the vegetable EPSPS activity together with the PAT and CRY1F proteins. Furthermore, the genes and their gene products have a history of safe use

through handling and consumption. Products and derivatives of the maize lines expressing these genes 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 request.

There are no wild relatives of maize in the Netherlands and the appearance of volunteers is very rare under Dutch conditions. 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 1507 x NK603 in the Netherlands will pose no risk.

The inserted genes and their gene products possess 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 these considerations, COGEM is of the opinion that the proposed import and processing for use in feed and food of the hybrid maize line 1507 x NK603 pose a negligible risk to human health and the environment.

References

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