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Onderwerp
Advies Marktdossier EFSA/GMO/UK/2004/06
Insectenresistente en herbicidentolerante maïs MON863 x NK603

Geachte heer Van Geel,

Naar aanleiding van het dossier EFSA/GMO/UK/2004/06, 'MON863 x NK603' voor 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 mogelijke risico's voor mens en milieu betreffende import en verwerking van een genetisch gemodificeerde (gg) maïslijn (kruising tussen de gg lijn MON863 en NK603). Teelt van deze maïslijn maakt geen deel uit van de vergunningaanvraag. Door insertie van het *cry3Bb1* gen, in de ouderlijn MON863 is resistentie voor keverachtige insecten, waaronder de maïswortelkever, verkregen. Hiernaast is de maïslijn door insertie van het *cp4 epsps* gen in NK603, tolerant geworden voor glyfosaat bevattende herbiciden.
Maïs kent 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 geen redenen om aan te nemen dat de modificatie het verwilderingspotentieel vergroot. Hiernaast is aangetoond dat de ingebrachte sequenties stabiel geïntegreerd zijn in de ouderlijnen en 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 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,

Prof. dr. ir. B.C.J. Zoeteman
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 NK603

COGEM advice: CGM/050228-03

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 MON863 and NK603. These parental lines contain genes (cry3Bb1 and cp4 epsps) conferring resistance to certain coleopteran insects, and tolerance to herbicides containing the active ingredient glyphosate.

Maize line MON863 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 run wild. Therefore, COGEM is of the opinion that incidental spillage of the hybrid maize line will pose no risk to the environment in the Netherlands.

Based on the southern blot analysis provided by the applicant, the experts of COGEM are of the opinion that the integration patterns of the introduced genes in the parental maize lines remain stable and unchanged in the upcoming generations. In addition, 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.

Considering the above-mentioned, COGEM is of the opinion that the proposed import and processing of the maize line MON863 x NK603 does not pose a significant risk for 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 MON863 x NK603 is developed through traditional breeding methods by crossing the genetically modified maize lines MON863 and NK603. Thereby, MON863 x NK603 inherits and effectively combines the two single-traits of the parental maize lines. The MON863 line is modified by the introduction and expression of the gene *cry3Bb1* that confers resistance to certain coleopteran insect pests, like the larvae of the corn rootworm (*Diabrotica* spp.). 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). MON863 is waiting for approval by the European environmental counsel.

Maize line MON863 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 MON863 for import and processing (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 (1,3). 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 (MON863 and NK603). The characterization of these 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. A gene fragment containing the *cry3Bb1* and the *nptII* expression cassette was inserted into the maize variety. The *cry3Bb1*gene confers resistance to coleopteran insects like

the corn root worm. By inserting the *nptII* gene, the plant acquires tolerance for kanamycin.

An overview of the introduced sequences is given below:
• *cry3Bb1* expression cassette
- 4AS1, promotor derived from the *Cauliflower mosaic virus* (CaMV); 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 induces the polyadenylation

• *nptII* gene cassette:
- 35S promotor, derived from 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 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 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

MON863 was genetically modified with the *cry3Bb1* gene derived from *B. thuringiensis* (subsp. *Kumamotoensis*). The produced Cry3Bb1, a δ-endotoxin, is lethal to insects of the *Coleoptera* order, including larvae of the corn rootworm (*Diabrotica* sp). 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 within 48 to 72 hours (5).

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 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. 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 as a result of (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.

Properties of the introduced genes conferring 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 the experts 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 and NPTII accumulate in the cytoplasm, while CP4 EPSPS is directed to the chloroplasts. 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 MON863 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 the above-mentioned, COGEM is of the opinion that the proposed import and processing for use in feed and food of the hybrid maize line MON863 x NK603 pose a negligible risk to human health and the environment.

References

(1) Hin CJA (2001). Rapport Landbouwkundige risico's van uitkruising van GGO-gewassen Centrum voor Landbouw en Milieu (CLM).

(2) Coe EHJR, Neuffer MG, Hoisington DA 1988. The genetics of Corn. pp. 81-258. In: Sprangue GF, Dudley JW, Editors. Corn and Corn Improvement, Third Edition. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, Wisconsin. 986 pp.

(3) Treau R and Emberlin J (2000). Pollen dispersal in the crops Maize (*Zea mays*), Oil seed rape (*Brassica napus* ssp. *Oleifera*), Potatoes (*Solanum tuberosum*), Sugar beet (*Beta vulgaris* ssp. *vulgaris*) and Wheat (*Triticum aestivum*)- Evidence from publications. Soil Association.

(4) Luna, V.S., Figueroa, M.J., Baltazar, M.B., Gomez, L.R., Townsend, R. and Schoper J.B. (2001). Maize pollen longevity and distance isolation requirements for effective pollen control. Crop Science **41**: 1551-1557.
(5) University of Florida. *Bt* (*Bacillus thuringiensis*), A microbial insecticide. Internet: http://miami-dade.ifas.ufl.edu/programs/urbanhort/publications/PDF/bt.pdf (17-2-2005)
(6) Het LNV-loket. Internet: http://www.hetlnvloket.nl/servlet/page?_pageid=274&_dad=portal30&_schema=PORTAL30&p_siteid=33&p_itemid=206020 (17-2-2005).
(7) Animal Science Group, Wageningen UR. Internet: http://www.pv.wageningen-ur.nl/index.asp?nieuws/nieuwbijpv/persberichten/2004080901.asp (17-2-2005).
(8) Della-Cioppa, G.S., Bauer, C., Klein, B.K., Shah, D.M., Fraley, R.T. and Kishore G.M. (1986). Translocation of the precursor of 5-*enol*pyruvylshikimate-3-phosphate synthase into chloroplasts of higher plants *in vitro*. Proceedings of the National Academy of Sciences **83**:6873-6877