

Aan de minister van
Volkshuisvesting, Ruimtelijke
Ordening en Milieubeheer
Mevrouw dr. J.M. Cramer
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DATUM 8 maart 2007
KENMERK CGM/070308-02
ONDERWERP Advies Cultivation of genetically modified maize line NK603 x MON810

Geachte mevrouw Cramer,

Naar aanleiding van een adviesvraag betreffende de teelt van genetisch gemodificeerde maïs NK603 x MON810 van Monsanto Europe S.A, deelt de COGEM u het volgende mee.

Samenvatting:

De COGEM is gevraagd te adviseren over de vergunningaanvraag voor de teelt van de genetisch gemodificeerde maïslijn NK603 x MON810. De maïslijn bevat het gen *cp4epsps* waardoor de plant tolerant is voor herbiciden met als werkzame stof glyfosaat. Daarnaast bevat de maïslijn het gen *cryIAb* waardoor de plant resistent is voor bepaalde insecten uit de orde van de Lepidoptera, zoals de Europese maïsboorder (*Ostrinia Nubilalis*).

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 is verder van mening dat de moleculaire karakterisering van de maïslijn volledig is. Daarnaast acht de COGEM het aannemelijk dat er geen schadelijke effecten op niet-doelwitorganismen te verwachten zijn.

De COGEM is van mening dat de risico's voor mens en milieu bij markttoelating voor teelt van de maïslijn NK603 x MON810 verwaarloosbaar klein zijn. De COGEM plaatst daarnaast nog een aantal opmerkingen bij het ontbreken van een aantal onderzoeksrapporten met betrekking tot niet-doelwitorganismen waarnaar verwezen wordt in deze aanvraag.

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

Hoogachtend,

A handwritten signature in black ink, consisting of a large, stylized loop on the left and a long, horizontal stroke extending to the right.

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

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

Cultivation of genetically modified maize line NK603 x MON810

COGEM advice CGM/070308-02

This notification concerns the cultivation of genetically modified maize line NK603 x MON810. The maize line harbors the genes cp4epsps and cry1Ab conferring tolerance to glyphosate containing herbicides and resistance to certain lepidopteran insects such as the European corn borer (Ostrinia Nubilalis).

Previously, COGEM advised positively on maize lines with the traits cp4epsps and cry1Ab. In 2003, COGEM issued a positive advice on the import and cultivation of parental maize line NK603 which expresses the cp4epsps gene.

Furthermore, COGEM advised positively on the import and cultivation of the parental maize line MON810 which expresses the cry1Ab gene. COGEM also advised positively on the import of the hybrid maize line NK603 x MON810.

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.

In the opinion of COGEM, the applicant has sufficiently proven that no toxic or allergenic products are formed as a result of the crossing of maize lines NK603 x MON810. In the opinion of the COGEM, the risk assessment is adequate and the post-market monitoring plan is sufficient.

COGEM wants to point out that the applicant refers to certain studies on non-target organisms which are not present in the dossier. However, COGEM already advised positively on the maize lines MON810 and Bt11. Bt11, MON810 and NK603 x MON810 show similar levels of Cry1Ab expression. COGEM concluded that it was sufficiently proven that the effects on non-target organisms are negligible. Based on these facts and the summary of the studies to which the applicant refers to, COGEM is of the opinion that possible effects on non-target organisms are negligible for maize line NK603 x MON810.

Based on these considerations, COGEM is of the opinion that the cultivation of maize line NK603 x MON810 poses a negligible risk to human health and the environment.

Introduction

The scope of the present notification by Monsanto Europe S.A concerns cultivation of hybrid maize line NK603 x MON810. The hybrid maize line NK603 x MON810 was

produced by crossing single trait NK603 and MON810 parents using traditional breeding methods.

The hybrid maize line expresses the genes *cp4epsps* and *cryIAb* conferring tolerance to glyphosate containing herbicides and insect resistance to certain lepidopteran insects such as the European corn borer (*Ostrinia Nubilalis*).

During the last few years, COGEM was asked repeatedly to issue advice on applications concerning the cultivation of various genetically modified (gm) maize varieties. To obtain permission for cultivation of a gm maize line, an environmental risk assessment has to be carried out by the applicant. The objective of the risk assessment is to identify and evaluate potential adverse effects (direct and indirect, immediate or delayed) of the genetically modified maize line on human health and the environment.

Environmental risk analyses focuses amongst others on the potential of the gm maize variety to establish feral populations; its potential to outcross with wild relatives and the effects of outcrossing on the environment; its potential to cause effects on non-target organisms and its potential to cause effects on the soil ecosystem and risks associated with incidental consumption by humans and animals.

To identify and evaluate these points, the crop characteristics, the molecular characterization of the gm plant (e.g. location of the insert and characteristics of the inserted genes) and the environment in which the plant is introduced (e.g. wild relatives, geographical and climatological conditions), are taken into account.

In case of maize, COGEM has repeatedly stated that maize is not able to run wild in the Netherlands, and that no wild relatives are present in Europe.

Previous COGEM advices

In 2003 COGEM advised positively on the import and processing of the parental line NK603 for use in feed and food (11). Three years later, COGEM also issued a positive advice on the cultivation of NK603 (12). As a consequence of the expression of the *cp4epsps* gene, NK603 is tolerant to glyphosate containing herbicides.

In the past COGEM has also issued advice on two maize varieties expressing the *cryIAb* gene. In 1995, COGEM advised positively on the cultivation of maize line MON810, expressing the *cryIAb* gene (13). Two years ago, in 2005, COGEM also gave a positive advice on the cultivation of maize line Bt11 (14).

Furthermore, COGEM advised positively on the import and processing of the hybrid maize line NK603 x MON810 (15). The current application concerns the cultivation of hybrid maize line NK603 x MON810.

Maize line NK603 x MON810

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 dormancy, resulting in a short persistence. 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.

Molecular characterization

The hybrid maize line NK603 x MON810 was produced by crossing single trait NK603 and MON810 parents using traditional breeding methods.

An overview of the introduced sequences is given below:

Components of the insert in NK603

- First cp4epsps expression cassette:
 - P-ract1/ ract1 intron, promoter and intron derived from *Oryza sativa*, intron promotes transcription
 - *ctp2* gene from *Arabidopsis thaliana*; encoding the N-terminal chloroplast transit peptide, which directs cp4 epsps protein to the chloroplast
 - *cp4epsps* gene from *Agrobacterium tumefaciens strain CP4*; encodes glyphosate tolerant CP4epsps protein
 - NOS 3', originating from *A. tumefaciens*, ends transcription and directs polyadenylation of the mRNA

- Second cp4epsps expression cassette:
 - e35S, promoter, originating from the *Cauliflower mosaic virus*
 - *Zmhsp70* gene, stabilizes the level of gene transcription
 - *ctp2* gene from *Arabidopsis thaliana*; encoding the N-terminal chloroplast transit peptide
 - *cp4epsps l214p*; encodes glyphosate tolerant CP4epsps l214p protein

- NOS 3', originating from *A. tumefaciens*, ends transcription and directs polyadenylation of the mRNA.

Components of the insert in MON810

- e35S, promoter, originating from the *Cauliflower mosaic virus*
- *Zmhsp70* gene, stabilizes the level of gene transcription
- *Cry1Ab*, DNA sequence originating from *Bacillus thuringiensis* encodes for a Cry1Ab protein.

Properties of the introduced genes conferring herbicide tolerance

Maize line NK603 was genetically modified with 2 *cp4epsps* genes encoding EPSPS. *Cp4epsps* encodes for Cp4epsps proteins possessing a high tolerance to glyphosate.

Epsps is a natural occurring enzyme involved in the biosynthesis of aromatic amino acids. In non-transgenic maize lines, glyphosate, an active ingredient of some herbicides, acts by binding to and 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 the glyphosate because of a reduced binding affinity. Because the hybrid maize line NK603 x MON810 expresses the *cp4epsps* gene, it has acquired a high tolerance to glyphosate. The application of this herbicide will not cause death of maize line NK603 x MON810, 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 *cp4epsps* gene, resulting in the transport of the transgenic Cp4epsps protein from the cytoplasm to the chloroplast (7).

Properties of the introduced genes conferring insect resistance

MON810 was genetically modified with the *cry1Ab* gene derived from *B. thuringiensis* (subsp. *Kumamotoensis*). The produced Cry1Ab, a δ -endotoxin, is lethal to insects of the lepidopteran order, including larvae of the European corn borer (*Ostrinia Nubilalis*) and the pink borer (*Sesamia cretica*). The δ -endotoxin selectively binds to receptors located in the midgut of susceptible insects. Following binding, 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 (6;10).

The larvae of the European corn borer cause severe damage to corn crops by feeding of 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, 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

The molecular characterization of maize NK603 x MON810 was previously evaluated by COGEM in an application concerning import and processing for the use in feed and food of maize line NK603 x MON810. It was concluded that the molecular characterization was adequate.

The applicant provided information, based on southern blot analysis, which shows that the integration patterns of the introduced genes in the line remain stable and unchanged in the upcoming generations. In the opinion of COGEM, the applicant had sufficiently proven by extensive bioinformatic analysis that no products are formed that show homology with known toxins or allergens.

To the opinion of the COGEM, interactions between recombinant gene products are not expected in the maize line because the gene products accumulate in different cellular compartments. The protein Cry1Ab accumulates in the cytoplasm, while Cp4epsps is directed to the chloroplasts.

Environmental risk assessment

In the opinion of COGEM, the risk is negligible that NK603 x MON810 becomes environmentally persistent or invasive resulting in unintended spreading through increased weediness.

Concerning the effects of Cry1Ab on non-target organisms, COGEM notices that the applicant refers to a series of studies which are only partially enclosed in the dossier. Consequently, the presented summary of the results of studies on non-target organisms can not be verified.

However, COGEM has issued an advice on two other maize varieties expressing the *cry1Ab* gene in the past. COGEM advised positively on the cultivation of maize line MON810 and maize line Bt11. These lines have a similar level of expression of Cry1Ab protein as the hybrid maize line in the current application. In approved maize variety Bt11, the effects on non target organisms were studied and no adverse effects were found. COGEM concluded that the applicant had sufficiently proven that the effects on non-target organisms are negligible. Since the current application, concerning the crossing of

maize lines NK603 x MON810 has similar expression levels of *cry1Ab*; it can be assumed that there will be no adverse effects on non-target organisms.

Products and derivatives of approved maize lines expressing the *cry1Ab* genes have been handled and consumed without reports of side-effects. Based on the history of safe use of maize lines MON810 and Bt11 and the similarities in *cry1Ab* expression levels, COGEM is of the opinion that NK603 x MON810 is not likely to exert negative effects on non-target organisms as a result of the crossing of the two parental maize lines NK603 and MON810.

Post-market monitoring plan

To obtain a permission to cultivate gm maize, a monitoring plan considering the environmental impact of cultivation is required. The applicant has formulated a general surveillance plan for NK603 x MON810 maize in order to detect any unanticipated adverse effects on human health and the environment. Key stakeholders and key networks will be requested to inform the applicant in case of potential occurrence of any unanticipated adverse effects to health or the environment. Furthermore, a number of farmers will be requested to participate in environmental surveys through questionnaires.

The general surveillance reports will be used to inform the European Commission on an annual basis. In case of the development of any adverse effects arising from handling and use of maize NK603 x MON810, the applicant will inform the European Commission immediately.

Considering the surveillance plan, COGEM agrees with the applicant that adverse effects are likely to be observed timely and consequently appropriate measures can be taken. COGEM is of the opinion that the surveillance plan is to a large extent sufficient.

However, COGEM notices that the general surveillance plan lacks detailed information on exactly which networks will inform the applicant in case of potential occurrence of any unanticipated adverse effects to human health or the environment and how this information will be communicated

Advice

The present application concerns the cultivation of maize line NK603 x MON810. NK603 x MON810 expresses the genes *cp4epsps* and *cry1Ab*, providing the plant with a herbicide tolerance trait and resistance to certain insects from the lepidopteran order. In the past, COGEM advised positively on maize lines with these traits and hybrid maize lines with both traits for import and processing as well as for cultivation.

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.

COGEM is of the opinion that the molecular characterization is adequate. In the opinion of COGEM, the risk assessment and the post-market monitoring plan are both adequate. Although the applicant did not provide all the underlying studies on non-target organisms, COGEM is of the opinion that no harmful effects are to be expected on non-target organisms. This is based on a comparison of Cry1Ab expression levels and studies on non-target organisms of Cry1Ab expressing maize lines MON810 and Bt11 which were approved for cultivation in the past.

In addition, single trait maize lines NK603 and MON810 are already approved for cultivation and have a history of safe use. No adverse effects on human health and the environment are reported.

In view of these considerations, COGEM is of the opinion that the proposed cultivation of maize line NK603 x MON810 poses a negligible risk to human health and the environment.

COGEM stresses that this positive advice on the hybrid Cry1Ab maize line concerns this specific case only and is not applicable to every *cry1Ab* event, since an event specific analysis is essential to provide a proper assessment on the risks for human health and the environment

Additional remarks

In the paragraph on environmental risk assessment, COGEM has already drawn attention to the fact that the dossier lacks certain studies on non-target organisms to which the applicant refers. The results of experiments referring to studies which are not available can not be verified and are not of any value in the assessment. In the past, COGEM has repeatedly made additional remarks on the quality of the presented dossiers in general.

COGEM points out that it is the responsibility of the applicant to provide complete and thorough information on a certain event. In case information concerning the molecular analysis or the environmental risk assessment is lacking, COGEM wants to underline that it is likely that no positive advice on the application can be given. In general, it is not possible to perform a proper risk assessment in case information or studies on this subject are lacking.

In this specific case, COGEM was able to gather information and examine similar cases of *cry1Ab* expressing maize lines. Maize lines MON810, which is one of the parental lines of the crossing in this application, and Bt11 appeared to express similar levels of the Cry1Ab protein. Therefore, COGEM considers it plausible that the effects

on non-target organisms are also similar and concluded that no adverse effects are expected on this aspect for the crossing of NK603 x MON810.

Furthermore, expected effects on non-target organisms are case specific and can not be reduced to the safety of a protein, such as Cry1Ab, in general. In the advise on Bt11 (CGM/050816-01), COGEM already made an additional remark on this subject. COGEM pointed out that especially dose-response studies are important to assess the degree of effect of the toxin.

Also, COGEM wants to draw attention to the scope of the studies on non-target organisms in this dossier. Most of the studies on non-target organisms appear to be based on naturally occurring species of the Lepidoptera order in the U.S.A. Although no adverse effects on non-target organisms in Europe are expected, the results are not directly applicable to European circumstances.

Finally, COGEM is of the opinion that presenting a dossier that refers to studies that are not available does not contribute to a transparent scientific and societal discussion on the assessment of gm organisms.

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