COMMISSIE
COGEM
GENETISCHE
MODIFICATIE

Aan de Staatssecretaris van
Volkshuisvesting, Ruimtelijke
Ordening en Milieubeheer
De heer drs. P.L.B.A. van Geel
Postbus 30945
2500 GX Den Haag

DATUM 4 juli 2006
KENMERK CGM/060704-01
ONDERWERP Teelt van maïslijn NK603 (EFSA/GMO/NL/2005/22)

Geachte heer Van Geel,

Naar aanleiding van het dossier EFSA/GMO/NL/2005/22 voor toelating van teelt van de genetisch gemodificeerde maïslijn NK603 van Monsanto Company adviseert de COGEM als volgt.

Samenvatting:
De COGEM is gevraagd te adviseren over de mogelijke risico's voor mens en milieu betreffende teelt van de genetisch gemodificeerde maïslijn NK603. De lijn is tolerant voor glyfosaat bevattende herbiciden door insertie van de genen *cp4 epsps* en *cp4 epsps L214P*.

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. Tevens zijn er geen redenen om aan te nemen dat de modificatie het verwilderingspotentieel vergroot.

Eerder heeft de COGEM, in het kader van een vergunningaanvraag voor import en verwerking, positief geadviseerd over de moleculaire karakterisatie van de maïslijn. Verder heeft de COGEM de milieurisico-analyse en het post-market monitoring plan beoordeeld en beide adequaat bevonden.

Maïslijn NK603 wordt al commercieel geteeld in de Verenigde Staten en heeft een geschiedenis van veilig gebruik.

Gezien het bovenstaande, acht de COGEM de risico's voor mens en milieu bij teelt van onderhavige maïslijn verwaarloosbaar klein.

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

c.c. Dr. ir. B.P. Loos
Dr. R.C. Zwart

Cultivation of herbicide tolerant maize line NK603

COGEM advice CGM/060704-01

This notification concerns the cultivation of genetically modified maize line NK603. The maize line harbors the genes cp4 epsps and cp4 epsps L214P conferring tolerance to herbicides containing the active ingredient glyphosate.

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.

As part of the notification for import and processing, COGEM already advised positively on the molecular characterization of NK603 in 2003. In the opinion of the COGEM, the risk assessment is adequate and the post-market monitoring plan is sufficient.

Maize line NK603 is commercially grown in the U.S.A. and has a history of safe use. No adverse effects on human health and the environment are reported.

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

1. INTRODUCTION

The scope of the present notification by Monsanto Company concerns the cultivation of maize line NK603. The maize line contains and expresses the genes *cp4 epsps* and *cp4 epsps L214P* conferring tolerance to glyphosate containing herbicides.

In the EU, NK603 has been approved for import and for use in food and feed (1;2). Since 2000, maize line NK603 is commercially grown in the USA (3). There are no reports of adverse effects on human health and the environment.

During the last few years, COGEM was asked repeatedly to issue advice on applications concerning the commercial import and processing of various genetically modified (gm) maize variants. Environmental risk analyses focus on 1) the potential of the gm maize variety to establish feral populations, 2) its potential to outcross with wild relatives and the effects of outcrossing on the environment, 3) its potential to cause effects on non-

target organisms and 4) risks associated with incidental consumption by humans and animals. Therefore, 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 the 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.

1.1 Previous COGEM advices

In the past, COGEM advised positively on importation and processing of maize line NK603 (CGM/030319-08). COGEM also advised positively on the commercial import, processing in feed and food and cultivation of maize lines 1507 x NK603 (CGM/050526-01 en CGM/060510-01). In addition, COGEM advised positively on importation and processing of hybrid maize lines MON810 x NK603, MON863 x NK603 en MON863 x MON810 x NK603 (CGM/040421-01, CGM/050228-03 en CGM/050526-01).

2. MAIZE LINE NK603

2.1 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 (4;5). According to literature, pollen viability varies between 30 minutes and 9 days (5;6;7). In Europe, no wild relatives of maize are present and, therefore, hybridisation 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 (4). 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 establish feral populations.

2.2 Molecular characterisation

The genetically modified maize line NK603 was produced by particle bombardment. A restriction fragment of plasmid PV-ZMGT32L, containing two *cp4 epsps* expression cassettes was inserted into the plant. CP4 EPSPS proteins confer tolerance to herbicides containing the active ingredient glyphosate.

The two expression cassettes contain the following sequences:

• *cp4 epsps* expression cassette 1:

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

• *cp4 epsps* expression cassette 2:

- E35S, constitutive promotor from *Cauliflower mosaic virus*
- *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 CP4 EPSPS
- Nos 3', terminator from *A. tumefaciens*; stops transcription

Properties of the introduced genes conferring herbicide tolerance

The genes *cp4 epsps* and *cp4 epsps L214P* are present in maize line NK603. These genes encode for CP4 EPSPS 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 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, CP4 EPSPS is not affected by glyphosate because of a reduced binding affinity. Because NK603 expresses the genes *cp4 epsps* and *cp4 epsps L214P*, it has acquired a high tolerance to glyphosate. The application of this herbicide will therefore not cause death of maize line NK603, because the plant is still able to produce aromatic amino acids (8).

Molecular analysis

The molecular characterization of maize NK603 was previously evaluated by COGEM. It was concluded that the molecular characterization was adequate. Analysis of the flanking regions of the NK603 insert demonstrated that the 3' flanking sequence of the insert is chloroplast genomic DNA. Most likely, the genomic chloroplast DNA co-integrated with the transgenic insert DNA during the transformation process. It is possible that the integration of the chloroplast sequence results in the rise of new chimeric open reading frames (ORFs) and consequently new proteins. To exclude the expression of novel proteins with unforeseen properties, the sequence of the six reading frames that span the junction of the transposed chloroplast DNA and plant genomic DNA was determined. Of the putative ORFs only two, respectively 25 and 47 amino acids in length, contain a

methionine residue. After extensive analysis, it was concluded that putative proteins encoded by the two ORFs show no sequence homology to known allergens or toxins.

2.3 Environmental risk assessment

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. It includes among others the evaluation of effects of the gm maize line on populations of target and non-target organisms, of the potential for gene transfer to the same or other sexually compatible plant species and of the likelihood of the maize line becoming more persistent than the recipient plants. Because of this thorough evaluation of adverse effects, COGEM is of the opinion that the risk assessment is adequate and that no adverse environmental effects are to be expected. Among others the risk is negligible that NK603 becomes environmentally persistent or invasive resulting in unintended spreading through increased weediness. In addition, there is no reason to assume that the maize line has a selective advantage resulting from the modification. Furthermore, NK603 is not likely to exert negative effects on non-target organisms as a result of the modification.

2.4 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 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 and to inform the applicant. 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, the applicant will inform the European Commission immediately. Considering the surveillance plan, COGEM is of the opinion that it is sufficient because adverse effects are likely to be observed timely and consequently appropriate measures can be taken.

COGEM agrees with the statement of the applicant that no potential hazards are identified from the environmental risk assessment. This conclusion is derived from

results of scientific studies (which were included in the dossier); therefore a case-specific monitoring plan is not necessary according to directive 2001/18/EC.

3. ADVICE

The present application concerns the cultivation of maize line NK603. In the past, COGEM advised positively on the import and processing of this line. COGEM also advised positively on cultivation of the hybrid maize line 1507 x NK603.

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 establish feral populations.

In the opinion of COGEM, the risk assessment is adequate and the post-market monitoring plan is sufficient. COGEM is of the opinion that the molecular characterization is adequate and that it is sufficiently proven that any putative novel proteins do not share homology with known allergens or toxins. In addition, maize line NK603 is commercially grown in the U.S.A. and has 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 poses a negligible risk to human health and the environment.

REFERENCES

(1) European commission (2004). Genetically modified NK603 maize authorised for both food and feed. Press release. Internet: http://europa.eu.int/rapid/pressReleasesAction.do?reference=IP/04/1305&format=HTML&aged=0&language=EN&guiLanguage=en (d.d. 13 juni 2006).

(2) European commission (2006). Newly authorised products. Internet: http://europa.eu.int-/comm/food/dyna/gm_register/index_en.cfm (d.d. 13 juni 2006).

(3) Agbios database product description. Internet: www.agbios.com. (d.d. 13 juni 2006).

(4) Hin, C.J.A. (2001). Rapport Landbouwkundige risico's van uitkruising van GGO-gewassen Centrum voor Landbouw en Milieu (CLM).

(5) 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.

(6) Coe, E.H.J.R., Neuffer, M.G. and Hoisington, D.A. (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.

(7) 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.

(8) Dill, G.M. (2005). Glyphosate-resistant crops: history, status and future. Pest Manag Sci 61: 219-224.