COMMISSIE
COGEM
GENETISCHE
MODIFICATIE

Aan de Staatssecretaris van
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Ordening en Milieubeheer
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KENMERK CGM/060510-01
ONDERWERP EFSA/GMO/UK/2005/17: maïslijn 1507 x NK603

Geachte heer Van Geel,

Naar aanleiding van het dossier EFSA/GMO/UK/2005/17 voor toelating van teelt van de genetisch gemodificeerde maïslijn 1507 x NK603 van Pioneer Hi-Bred International, Inc. en Mycogen Seeds, adviseert de COGEM als volgt.

Samenvatting:
De COGEM is gevraagd te adviseren over de mogelijke risico's voor mens en milieu betreffende teelt van een genetisch gemodificeerde maïslijn (kruising tussen de lijnen 1507 en NK603). De ouderlijn 1507 heeft door insertie van het *cry1F* gen en het *pat* gen respectievelijk resistentie tegen vlinders en motten, waaronder de Europese maïsboorder, en tolerantie voor herbiciden met als werkzame stof glufosinaat-ammonium, verkregen. De ouderlijn NK603 is tolerant voor glyfosaat bevattende herbiciden door insertie van het *cp4 epsps* gen. In de kruisingslijn worden de drie eigenschappen gecombineerd.

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. De COGEM heeft het post-market monitoring plan beoordeeld en dit adequaat bevonden. Eerder heeft de COGEM de moleculaire karakterisatie van de hybride maïslijn al positief beoordeeld.

Maïslijn 1507 x NK603 wordt al commercieel geteeld in de Verenigde Staten en heeft een geschiedenis van veilig gebruik. Daarnaast heeft de COGEM eerder al positief geadviseerd over de beide ouderlijnen.

Gezien het bovenstaande, acht de COGEM de risico's voor mens en milieu bij teelt van onderhavige maïslijn verwaarloosbaar klein. Tevens signaleert de COGEM dat de studies naar effecten op niet-doelwitorganismen meer gericht zouden moeten zijn op de Europese situatie. Daarnaast is het gewenst dat criteria waaraan deze studies moeten voldoen, worden gestandaardiseerd.

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 insect resistant and herbicide tolerant maize 1507 x NK603

COGEM advice CGM/060510-01

*The present application concerns the cultivation of a genetically modified maize line. 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.*

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.

COGEM evaluated the post-market monitoring plan and concluded that is was adequate. In the past, COGEM advised positively on the molecular characterization of maize 1507 x NK603.

Maize line 1507 x NK603 is already commercially grown in the USA and has a history of safe use. In the past, COGEM advised positively on both parental maize lines 1507 and NK603.

Based on these considerations, COGEM is of the opinion that the cultivation of maize line 1507 x NK603 poses a negligible risk to human health and the environment. Furthermore, COGEM stresses the importance of a relevant and thorough study on the effects of non-target organisms. COGEM urges that EU-wide, standardized criteria are formulated.

1. INTRODUCTION

The present application concerns the cultivation of the hybrid maize line 1507 x NK603. This line is developed through traditional breeding methods between the genetically modified (gm) maize lines 1507 and NK603. Therefore, 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, such as the larvae of the European corn borer (*Ostrinia nubilalis*), and to *Sesamia* spp. like the pink borer (*Sesamia cretica*). In addition, the

inserted *pat* gene confers tolerance to herbicides containing the active ingredient glufosinate-ammonium. The maize line NK603 contains and expresses the genes *cp4 epsps* and *cp4 epsps L214P*. These genes confer tolerance to glyphosate herbicides.

In the EU, both parental maize lines 1507 and NK603 have been approved for import and for use in food and feed (1;2;3).

Since 2001, maize line 1507 x NK603 is commercially grown in the USA (4). There are no reports of adverse effects on human health and the environment concerning handling and consuming products and derivatives of this line.

During the last few years, COGEM was asked repeatedly to issue advice on applications concerning the commercial import and processing of various gm maize variants. Environmental risk analyses focuses 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 has advised positively on the commercial import and processing in feed and food of maize line 1507 x NK603 (CGM/050526-01). COGEM also advised positively on both genetically modified parental maize lines NK603 and 1507 for import and processing (CGM/030319-08, CGM/030115-01). In addition, COGEM advised positively on the cultivation of maize line 1507, although COGEM was of the opinion that the quality of the studies that identify effects of the maize line on non-target organisms was incomplete (CGM/030919-04).

2. MAIZE LINE 1507 x 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 (5;6). According to

literature, pollen viability varies between 30 minutes and 9 days (6;7;8). 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. Grains exhibit no germination dormancy, resulting in a short persistence. In addition, only few seeds remain on the field after harvesting of fodder maize (5). 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 present stacked-trait maize line is established by crossing 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 corn 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 *Z. 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 from *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, promoter, transcription start site 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*; stops transcription

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 corn 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 for larvae of certain lepidopteran insects after ingestion.

The specificity of Cry1F is directly related to the presence of certain binding sites in the target insects. Mammalian intestinal cells lack such binding sites. Therefore, humans 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
Hybrid maize line 1507 x NK603 combines *pat* and *epsps* genes to confer 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 (9). Glutamine synthetase is also involved in ammonia detoxification. By inhibiting glutamine synthase, the glutamine level in the plant reduces and the concentration of ammonia in plant tissues increases. This leads to cell membrane disruption and termination of photosynthesis, resulting in plant withering and death.

Expression of the *pat* gene results in the catalysis of glufosinate acetylation which leads to the detoxification of glufosinate into an inactive compound (11).

Two *cp4 epsps* genes encoding EPSPS are present in the parental maize line NK603. In this way tolerance was obtained to glyphosate herbicides. Glyphosate inhibits the function of natural occurring vegetable 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 1507 x NK603, because the plant is still able to produce aromatic amino acids.

Molecular analysis

The molecular characterization of maize 1507 x NK603 was previously evaluated by the COGEM. The COGEM concluded that the molecular characterization was adequate and from the data could be concluded that there is no significant risk for human health and the environment. As was stated previously, interactions between recombinant gene products are not expected in the hybrid maize line according to the experts of the COGEM. 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 already combines 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 reported side-effects. It is sufficiently proven that no toxic or allergenic products are formed as a result of the crossing.

2.3 Environmental risk assessment

For cultivation of a gm maize line an environmental risk assessment should be carried out. 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 the evaluation of effects of the gm maize line on populations of target and non-target organisms and on the soil environment.

Based upon the environmental risk analysis of the application concerning cultivation of parental maize line 1507 and upon the EFSA opinion on line 1507, the applicant concludes that there is no anticipated adverse effect on human and animal health and on the environment (9). However, the EFSA states that there is a limited potential for development of resistance within the target pest population to CRY1F proteins as expressed in cultivated 1507 x NK603 maize (10).

2.4 Post-market monitoring plan

For the cultivation of gm maize a monitoring plan is required that considers the environmental impact of cultivation. As mentioned above, the only effect of cultivating maize line 1507 x NK603 acknowledged by the applicant, is the potential development of resistance to CRY1F within the target pest population. To ensure that cultivation of 1507 x NK603 maize poses negligible risks, the applicant has formulated a case-specific monitoring plan consisting of an insect resistance management plan (IRM plan). This plan includes the following elements:

1. Establishment of the baseline susceptibility of European corn borer (*O. nubilalis*) and Mediterranean corn stalk borer (*S. nonagrioides*) to CRY1F protein;
2. Detection of changes relative to baseline susceptibility that could result in inadequate protection against *O. nubilalis* and *S. nonagrioides*, in the field;
3. Proactive delay of the potential development of pest resistance to the CRY1F protein.

Furthermore, the applicant formulates a general surveillance plan for 1507 x NK603 maize in order to protect against any unanticipated adverse effects on human health and the environment. Relevant information on the studied parameters will be collected on a regular basis through a questionnaire. This includes guidance and reporting procedures when observing adverse effects of the hybrid maize line. The applicant will encourage growers using maize 1507 x NK603 to fill in the form shortly after the harvest period.

In case of development of any adverse effects arising form handling and use of maize 1507 x NK603, the applicant will inform the European Commission immediately. Moreover, the applicant will investigate the reports and inform the European Commission of the outcome. This applies to both the case-specific monitoring and the general surveillance.

2.5 Phytosanitary aspects

Damage to maize plants is mainly caused by feeding of the larvae of pest insects on the stalk or ear shank. Yield losses are largely attributable to a reduction in kernel number

and weight owing mainly to physiological disruption of the plant growth and only to a minor extent to broken stalks, dropped ears and larval feeding on the grain (12;13).

The pink borer is a pest insect in Southern and Central Europe. There are also reports of incidences in Germany. In the Netherlands, the climate is not optimal for this insect.

The European corn borer is a pest insect in the United States and Canada. In the European Union the European corn borer is an important pest insect in countries such as France, Austria, Germany, Italy, Spain, Greece and Portugal. 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. In addition, the Dutch climate is not optimal for the European corn borer.

3. ADVICE

The present application concerns the cultivation of hybrid maize line 1507 x NK603. Previously, COGEM advised on the cultivation of parental maize line 1507. COGEM noted that the quality of the studies which determine effects of the maize line on non-target organisms was incomplete. Furthermore, the performance of the studies was not consistent and was not focussed on the European situation. However, the overall opinion of COGEM was that the cultivation of maize 1507 poses no risks to human health and the environment in the Netherlands.

COGEM points out that the current application uses the same studies as in the application concerning the cultivation of maize line 1507. Although these studies are incomplete, based upon expert judgement, COGEM is of the opinion that with the cultivation of maize line 1507 x NK603 only minor effects on non-target organisms could occur. Moreover, spraying with currently used insecticides could cause larger effects.

COGEM stresses the importance of a relevant and thorough study on the effects of non-target organisms. COGEM realises that standardized criteria for the relevant studies are still lacking. Therefore, COGEM urges that EU-wide, standardized criteria are formulated. To facilitate this, COGEM has taken the first preliminary steps by commissioning a research project on the determination of effects of insect-resistant transgenic crops on non-target arthopods (15).

In view of the following arguments:

- maize line 1507 x 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;
- there are no reasons to assume that the inserted traits will increase the now absent potential of the maize line to establish feral populations;
- there are no wild relatives of maize in the Netherlands;
- the appearance of volunteers is very rare under Dutch conditions;
- the molecular characterization is adequate;
- the post-market monitoring plan is sufficient;
- COGEM advised positively on both parental maize lines 1507 and NK603.

Therefore, the overall opinion of COGEM is that cultivation of 1507 x NK603 poses a negligible risk to human health and the environment in the Netherlands.

3.1 Additional remarks

COGEM notes that the proposed monitoring approach will only be adequate in case of recessive inheritance of resistance. In the application, insect resistant monitoring is completely based on the assumption that Bt-resistance is a recessive trait and that only homozygous individuals will be resistant. However, a dominant resistance allele can immediately increase in frequency under selective circumstances, such as the presence of Bt-toxin. In addition, literature shows that Bt-resistance in the European corn borer can also be partly dominant (14). As insect resistance management is of agronomical importance and not part of the environmental risk analysis, this aspect is not included in this advice.

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