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 6 juni 2006
KENMERK CGM/060606-01
ONDERWERP EFSA/GMO/UK/2005/19: import en verwerking van maïslijn GA21

Geachte heer Van Geel,

Naar aanleiding van het dossier EFSA/GMO/UK/2005/19 betreffende de toelating van de genetisch gemodificeerde maïslijn GA21 voor import en verwerking van Syngenta Crop Protection AG, adviseert de COGEM als volgt.

Samenvatting:
De COGEM is gevraagd te adviseren over de import en verwerking van een genetisch gemodificeerde maïslijn (GA21). Teelt van deze lijn maakt geen deel uit van de vergunningaanvraag. In de maïslijn is het gen *epsps* ingebouwd waardoor de plant tolerant is voor herbiciden met als werkzame stof glyfosaat.
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 acht daarom de kans verwaarloosbaar klein dat incidenteel morsen leidt tot verspreiding van GA21 binnen Europa.
De COGEM is van mening dat de moleculaire karakterisering van de maïslijn onvolledig is. In het dossier ontbreken gegevens die duidelijkheid verschaffen over de 5' flankerende sequenties. Hierdoor kan niet volledig uitgesloten worden dat er ten gevolge van de insertie nieuwe open leesramen zijn ontstaan die theoretisch tot toxische of allergene producten kunnen leiden. Gezien de onvolledigheid van de gegevens kan de COGEM geen positief advies geven voor markttoelating van GA21.

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

Import and processing of herbicide tolerant maize GA21

COGEM advice CGM/060606-01

The present notification concerns the commercial import and processing for use in feed and food of a genetically modified maize line. The scope of the application excludes cultivation. Maize line GA21 contains the modified epsps gene conferring tolerance to herbicides containing the active ingredient glyphosate.

The notification comprehends importation and processing of maize. Therefore, release in the environment can only occur by spillage of maize kernels. In the Netherlands, no wild relatives of maize are present and the appearance of volunteers is never a problem 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. Consequently, COGEM is of the opinion that incidental spillage of the hybrid maize line will not pose a risk to the environment in the Netherlands. However, there is a lack of information regarding the molecular analysis. Incomplete data are presented on the 5' flanking sequence. On basis of the available data it can not be ruled out that new chimeric open reading frames were created due to the insertion. Theoretically, these putative open reading frames could give rise to potentially toxic or allergenic products.

Based on these considerations, COGEM can not advice positively on the import and processing of the maize line GA21.

Introduction

The scope of the present notification by Syngenta Crop Protection AG includes commercial import and processing for use in feed and food of the genetically modified (gm) maize line GA21. The maize line contains and expresses the modified gene *epsps* conferring tolerance to glyphosate containing herbicides. The cultivation of GA21 is not included in the scope of this notification and is therefore not addressed in this advice.

In the EU, maize line GA21 has been authorised for use as food and food ingredients (1). The maize line is currently commercially grown in the USA and Canada (2). 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 events.

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, and 3) risks associated with incidental consumption by humans and animals. Therefore, the crop characteristics, the molecular characterization of the GMO (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.

Previous advices

In the past, COGEM advised positively on a notification by Monsanto concerning commercial import and processing in feed and food of maize line GA21 (CGM/030120-02). The application was later withdrawn by Monsanto.

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 (3; 4). According to literature, pollen viability varies between 30 minutes and 9 days (4; 5; 6). 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 (3). 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.

Molecular characterisation

Transformation event GA21 was produced via microprojectile bombardment of maize suspension culture cells. By inserting a modified version of the gene *epsps* (*mepsps*), the plant acquires tolerance to herbicides containing glyphosate.

An overview of the introduced sequences is given below:

- Rice actin promoter and intron, derived from *Oryza sativa* L; promotes constitutive expression of *mepsps*
- optimised transit peptide sequence, based on sequences from Z. mays and *Helianthus annuus* L.; directing *mepsps* to the chloroplast

- modified *epsps*, gene derived from *Z. mays*; encoding 5-enolpyruvylshikimate-3-phophatesynthase (EPSPS)
- Nos 3', terminator from *A. tumefaciens*; ends transcription

Properties of the introduced genes conferring herbicide tolerance

Maize line GA21 was modified by insertion of the *mepsps* gene encoding EPSPS to obtain tolerance to glyphosate-based herbicides. Glyphosate inhibits the function of naturally 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. GA21 expresses a modified *epsps* gene which possesses a high tolerance to glyphosate. The application of glyphosate will therefore not cause death of maize line GA21, because the plant will still be able to produce aromatic amino acids (7).

Molecular analysis

A *Not*I restriction fragment harbouring the aforementioned genetic elements was used in the transformation process. The applicant shows that six, partly incomplete, copies of this fragment are present at the insertion site. Other copies of the insert elsewhere in the genome or backbone sequences are absent. Both the 5' and 3' flanking sequences of the insert have been determined and analyzed for the presence of putative open reading frames (ORFs). The analysis of the 3'end reveals no special circumstances. Two putative ORFs are identified at the 5'end of the insert. One of these ORFs appears to originate from the maize 5' sequence and to extent into the insert sequences. The other ORF is truncated or disrupted and ends at the insertion site. Sequence analysis shows that the putative proteins encoded by the ORFs do not share homologies with known allergens or toxins. COGEM is of the opinion that the data provided by the applicant provide conclusive evidence that the presence of the ORFs does not pose a risk to human health or the environment.

However, sequence analysis shows that the 5' flanking maize sequence is of chloroplast origin. Possibly this sequence was already present in the maize genome prior to the insertional event or, more likely, genomic chloroplast DNA co-integrated with the transgenic insert DNA during the transformation process. Apparently it was not determined whether the chloroplast DNA was already present in the parental maize line. Moreover, data are lacking concerning the length of the chloroplast sequences or the transition site between the chloroplast and genomic sequences.

Indeed, in case the chloroplast DNA co-integrated it can not be completely excluded that upstream of the chloroplast sequences other sequences were integrated. Moreover, it is possible that the integration of the chloroplast sequence or other putative sequences

results in the rise of new chimeric open reading frames at the insertion site by fusion of ORFs of transgenic origin and plant origin.
In view of these uncertainties COGEM is of the opinion that the applicants have to provide a more detailed study of the 5' flanking sequence extending beyond the chloroplast sequences.

Advice

The present application concerns importation and processing for feed and food use of the genetically modified maize line GA21. The scope of the notification excludes cultivation of the line.

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 GA21 will pose no risk to the environment in the Netherlands.

However, COGEM is of the opinion that the molecular characterisation of GA21 is incomplete. Data concerning the presence of chloroplast sequences at the 5'-end of the insert are incomplete. COGEM is of the opinion that these data are a prerequisite to exclude the potential for insertional events to produce any novel chimeric proteins with unforeseen properties. GA21 is commercially grown in the USA and Canada and used for food and feed purposes. It can be argued that the fact that no harmful effects have been reported is a strong indication that novel chimeric proteins with adverse effects are absent in GA21. On the other hand, data supporting a history of safe use are not provided. Information is lacking on the extent of cultivation or consumption, and whether a system is in place to report putative detrimental effects. Therefore, COGEM takes the view that all the requirements of a full molecular characterisation have to be met by the applicant.
Consequently, COGEM does not advise positively on this application for the commercial import and processing for use in feed and food of the genetically modified maize line GA21.

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