Commissie Genetische Modificatie

Voorzitter: prof.dr.ir. B.C.J. Zoeteman

Cogem
Posbus 578
3720 AN Bilthoven

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

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Onderwerp
Advies Marktdossier EFSA/GMO/UK/2005/11
Maïs MIR604

Geachte heer Van Geel,

Naar aanleiding van de adviesvraag betreffende het dossier EFSA/GMO/UK/2005/11, 'maïslijn MIR604' over de import en verwerking van genetisch gemodificeerde maïs door 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 (MIR604). Teelt van deze lijn maakt geen deel uit van de vergunningaanvraag. In de maïslijn zijn de genen c*ry3A* en *pmi* ingebouwd waardoor de plant minder gevoelig is voor de larven van de maïswortelkevers en daarnaast mannose kan gebruiken als enige koolstofbron.

De vergunningaanvraag betreft alleen import. De enige wijze waarop maïs in het milieu verspreid kan worden is door morsen van maïskorrels. 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 is geen reden om aan te nemen dat de modificaties het verwilderingspotentieel vergroten. De COGEM is gezien het bovenstaande van mening dat het incidenteel morsen van de genetisch gemodificeerde maïs in Nederland geen risico's voor het milieu met zich meebrengt.

De COGEM acht derhalve de risico's voor mens en milieu bij de import en verwerking 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. I. van der Leij

Title: Import and processing of maize variety MIR604 (EFSA/GMO/UK/2005/11)

COGEM advice: CGM/051122-02

The present application concerns the commercial import and processing for use in feed and food of a genetically modified maize line. Cultivation is not part of the application. The maize line MIR604 is genetically modified by the introduction and expression of the gene cry3A that confers resistance to certain coleopteran insects. The insertion and expression of the gene pmi (manA), allows MIR604 to utilize mannose as a sole carbon source.

The application only comprehends the import 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 establishment of maize plants in the wild has never been observed. 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 the hybrid maize line will not pose a risk to the environment in the Netherlands.

In view of the aforementioned, COGEM is of the opinion that the risks for the environment and human health associated with the import and processing of the maize line MIR604 are negligible.

Introduction

The present application of Syngenta Crop Protection AG concerns the commercial import and processing for use in feed and food of a genetically modified maize line.

The maize line MIR604 is genetically modified by the introduction and expression of the gene c*ry3A* that confers resistance to the Western corn rootworm (*Diabrotica virgifera virgifera*), the Northern Corn rootworm (*Diabrotica longicornis barberi*) and other related coleopteran species.

The insertion and expression of the gene *pmi* (*manA*) allows MIR604 to utilize mannose as a sole carbon source.

The use of the *cry3A* and the *pmi* genes have not been assessed before by the Netherlands with respect to safety for 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 focuses on 1) the potential

of the gm maize variety to establish feral populations, 2) its potential to outcross with wild relatives and 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 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, and that no wild relatives are present in Europe.

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;2). Pollen viability varies between 30 minutes and nine days according to literature (2;3;4). 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. Maize kernels exhibit no germination dormancy, resulting in a short persistence. Furthermore, during harvesting of fodder maize only few seeds remain on the field (1). 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 run wild.

Molecular characterisation

Origin and function of the introduced genes

Maize line MIR604 is genetically modified via Agrobacterium-mediated transformation. A modified version of the gene c*ry3A* (*mcry3A*) is introduced and confers resistance to the Western corn rootworm and Northern corn rootworm. The plant is capable of using mannose as a sole carbon source by inserting the *pmi* gene.

An overview of the introduced sequences is given below:

• Active ingredient cassette

- MTL promotor, derived from the *Zea mays* metallothionein-like gene; provides root-preferential expression
- *mcry3A* gene, from *Bacillus thuringiensis* subsp. *tenebrionis*; confers resistance to coleopteran insects

- NOS, terminator sequence from the nopaline synthase gene of *Agrobacterium tumefaciens*

• Selectable marker cassette:

- ZmUbiInt promotor, derived from the Zea mays polyubiquitin gene; provides constitutive expression in monocots
- *pmi* gene, from *Escherichia coli*; catalyzes the isomerization of mannose-6-phosphate to fructose-6-phosphate
- NOS, terminator sequence from the nopaline synthase gene of *Agrobacterium tumefaciens*

Properties of the introduced genes

Insect resistance

Insect resistance of the maize plant MIR604 is increased by insertion of the gene *mcry3A*. Additional changes were made to the gene *cry3A* to enhance the activity of the expressed protein against certain coleopteran pests, particularly the Western corn rootworm and the Northern corn rootworm.

The gene *cry3A* is derived from *B. thuringiensis* (subsp. *tenebrionis*). By inserting the genes, plants will produce δ-endotoxins (Bt-toxins). These toxins are insecticidal to larvae of certain coleopteran insects. The toxins selectively bind to receptors located in the midgut, resulting in gut perforation causing death of the insect within 48 to 72 hours (5).

The corn rootworm is an economically important pest insect, causing major crop losses. Larvae of this insect feed on 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.

The 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 a 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 Schiphol airport in the Netherlands, but eradicated successfully. In 2005, incidental outbreaks of this pest have been reported and eradicated (6;7). The COGEM notes that when the corn rootworm is able to establish and spread itself in the Netherlands, large damage to crops can be expected (8;9). Successful introduction by (air) traffic might occur anytime in the near future.

The Cry3A protein does not exhibit amino acid homology to known allergens or toxins.

Selectable marker

Maize line MIR604 was genetically modified with the gene *pmi* (*manA*) encoding for the enzyme phosphomannose isomerase (PMI). As a result of the gene insertion, plants are capable of using mannose as a carbon source. Mannose is phosphorylated to mannose-6-phosphate (M6P) which can be converted to fructose-6-phosphate with the help of PMI. In non-gm plants lacking PMI, conversion of M6P will not occur. M6P will accumulate, block glycolysis, and inhibit plant growth. Consequently, the insertion of *pmi* has led to the introduction of a selection system in MIR604. Mannose is used as the selective agent and is applied to cell cultures to select transformed cells. It is not used as a selective agent in mature plants.

The PMI protein does not exhibit amino acid homology homology to known allergens or toxins.

Molecular analysis

Based on southern blot analysis, the integration patterns of the introduced genes in the parental lines remain stable and unchanged in the upcoming generations. Furthermore, a careful calculation based on the detection limit of the southern blot system used and the probe and genome size indicated that no backbone sequence is present in maize variety MIR604.

The information provided by the applicant sufficiently proves that no novel ORF's potentially encoding new proteins or fusion proteins are present. Furthermore, identified genes in *Zea mays* do not appear to be disrupted by the insert.

Furthermore, the COGEM notes that the applicant has provided evidence that incidental consumption of Cry3A and PMI will not have adverse effects. In addition, interactions between the different recombinant gene products are not expected in the maize line because the proteins have a different mode of action.

Advice

The present application concerns the import, processing for feed and food use of a hybrid maize line. Cultivation of the present line is not part of the application.

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 MIR604 will pose no risk in the Netherlands.

In view of the above, COGEM is of the opinion that the risks for the environment and human health associated with the import and processing of the maize line MIR604 are negligible.

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