COMMISSIE
COGEM
GENETISCHE
MODIFICATIE

Aan de minister van
Volkshuisvesting, Ruimtelijke
Ordening en Milieubeheer
Mevrouw dr. J.M. Cramer
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DATUM 17 oktober 2008
KENMERK CGM/081017-03
ONDERWERP Advies import en verwerking maïslijn Bt11xMIR604xGA21 (EFSA/GMO/UK/ 2008/56)

Geachte mevrouw Cramer,

Naar aanleiding van de adviesvraag betreffende het dossier EFSA/GMO/UK/2008/56 voor de import en verwerking van de genetisch gemodificeerde maïslijn Bt11xMIR604xGA21 door Syngenta Seeds S.A.S. adviseert de COGEM als volgt:

Samenvatting:
De COGEM is gevraagd te adviseren over de toelating voor import en verwerking van maïslijn Bt11xMIR604xGA21. Deze maïslijn is tot stand gekomen door het kruisen van drie genetisch gemodificeerde ouderlijnen.
De COGEM heeft eerder positief geadviseerd over import en verwerking van de drie ouderlijnen en over teelt van ouderlijn Bt11. Daarnaast heeft de COGEM positief geadviseerd over import en verwerking van de kruisingslijnen Bt11xGA21, Bt11xMIR604 en MIR604xGA21.
Maïslijn Bt11xMIR604xGA21 brengt zowel het *cry1Ab* als het *mcry3A* gen tot expressie, waardoor de plant resistent is voor bepaalde insecten die behoren tot de orde van de Lepidoptera en voor bepaalde insecten die behoren tot de orde van de Coleoptera. Daarnaast bevat deze maïslijn de genen *pat* en *mepsps*, waardoor de plant tolerant is voor glyfosaat en glufosinaat-ammonium bevattende herbiciden. Ten slotte is de plant door expressie van het *pmi* gen in staat mannose als koolstofbron te gebruiken. Deze laatste eigenschap kan als selectiemarker gebruikt worden.
Verwildering van maïsplanten is in Nederland nooit waargenomen. Daarnaast is opslag van maïsplanten in Nederland nagenoeg uitgesloten. Er zijn geen redenen om aan te nemen dat expressie van de geïnserteerde genen het verwilderingspotentieel van maïs vergroot. Bovendien zijn er in Europa geen wilde verwanten van maïs aanwezig waardoor uitkruising niet mogelijk is.
Gezien het bovenstaande heeft de COGEM geen bezwaar tegen import en verwerking van maïslijn Bt11xMIR604xGA21 en acht zij de risico's voor het milieu 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. Drs. H.P. de Wijs
Dr. I. van der Leij

Import and processing of maize Bt11xMIR604xGA21

COGEM advice CGM/081017-03

Summary

The present application by Syngenta Seeds S.A.S. (file EFSA/GMO/UK/2008/56) concerns import and processing for use in feed and food of the genetically modified maize line Bt11xMIR604xGA21. Cultivation is not part of this application.

Maize line Bt11xMIR604xGA21 was obtained by conventional cross-breeding of the genetically modified maize lines Bt11, MIR604 and GA21. Previously, COGEM issued positive advices on applications for import and processing of all three parental maize lines, and on cultivation of maize line Bt11. In addition, COGEM advised positively on import and processing of hybrid maize lines Bt11xGA21, Bt11xMIR604 and MIR604xGA21.

Maize line Bt11xMIR604xGA21 contains the cry1Ab and mcry3A genes conferring resistance to certain lepidopteran and coleopteran insects. In addition, this maize line contains the genes pat and mepsps, resulting in tolerance to glyphosate and glufosinate ammonium containing herbicides. Finally, this maize line contains the pmi gene which acts as a selectable marker enabling transformed plant cells to utilize mannose as a carbon source.

During its long domestication process, maize has lost its ability to survive in the wild. In the Netherlands, the appearance of maize volunteers is rare and establishment of volunteers in the wild has never been reported. There are no reasons to assume that the introduced traits will increase the potential of maize to establish feral populations. The genes introduced in Bt11xMIR604xGA21 cannot spread to closely related species since wild relatives of maize are not present in Europe.

In view of the above, COGEM is of the opinion that incidental spillage of Bt11xMIR604xGA21 poses negligible risks to the environment. Therefore, COGEM considers the risks associated with import and processing of maize line Bt11xMIR604xGA21 negligible.

Introduction

The present application by Syngenta S.A.S., file EFSA/GMO/UK/2008/56, concerns the import and processing of maize line Bt11xMIR604xGA21 for use in feed and food. Maize line Bt11xMIR604xGA21 was obtained by conventional cross-breeding of the parental lines Bt11, MIR604 and GA21. Maize line Bt11xMIR604xGA21 contains the *cry1Ab, mcry3A, pat, mepsps* and *pmi* genes, which are constitutively expressed. As a result Bt11xMIR604xGA21 is resistant to certain lepidopteran and coleopteran insects, tolerant to glyphosate and glufosinate ammonium containing herbicides, and able to use mannose as a carbon source.

Previous COGEM advices

In 1997, COGEM issued a positive advice on import and processing of maize line Bt11 (1), and in 2008 COGEM advised positively on the renewal of this application (2). A positive advice on cultivation of this maize line has been issued in 2005 (3). In the same year, COGEM issued a positive advice on import and processing of maize line MIR604 (4). COGEM has also advised on maize line GA21 and concluded that the ecological risks associated with import and processing of this maize line are negligible (5, 6).

In 2008, COGEM issued positive advices on import and processing of hybrid maize lines Bt11xGA21, Bt11xMIR604 and MIR604xGA21 (7, 8, 9).

Aspects of the crop

Maize (*Zea mays*) is a member of the *Poaceae* family (grasses). Maize was domesticated in Central America and is nowadays cultivated throughout the world (10). In Europe, hybridization with other species cannot occur as wild relatives of maize are not present in Europe (10). The appearance of volunteers is very rare under Dutch conditions. Grains exhibit no germination dormancy, resulting in a short persistence. Establishment of maize plants in the wild has never been observed in the Netherlands. Besides, observations outside the Netherlands indicate that feral maize populations do not occur in Europe.

Molecular characterization

Maize line Bt11xMIR604xGA21 was produced by conventional cross-breeding of the genetically modified maize lines Bt11, MIR604 and GA21. Information on the elements introduced in these maize lines is given below. The molecular characterization of the maize lines has been discussed in previous advices on maize lines Bt11, MIR604 and GA21 and in previous advices on Bt11xGA21, Bt11xMIR604 and MIR604xGA21 (1-9).

<u>Maize line Bt11:</u>

Bt11 maize was generated by transformation of *Z. mays* protoplasts using a *Not*I restriction fragment which contains the *cry1Ab* and *pat* gene cassettes.

The *cry1Ab* gene cassette consists of the following elements:

- 35S promoter, derived from *Cauliflower mosaic virus* (CaMV)
- IVS6-ADH1 intron, intervening intron sequence 6 derived from the alcohol dehydrogenase 1 (*adh1*) gene of maize
- truncated *cry1Ab* gene, derived from *Bacillus thuringiensis* var. *kurstaki* HD-1, truncated at the 3' end and modified to enhance expression in plants. The Cry1Ab protein confers resistance to certain lepidopteran insects
- NOS terminator, derived from the nopaline synthase (*nos*) gene of *Agrobacterium tumefaciens*

The *pat* gene cassette consists of the following elements:

- 35S promoter, derived from CaMV
- IVS2-ADH1 intron, intervening intron sequence 2 derived from the alcohol dehydrogenase 1 (*adh1*) gene of maize
- *pat* gene, derived from *Streptomyces viridochromogenes* strain Tu494 and codon-optimized to enhance expression in maize. The *pat* gene encodes phosphinothricin acetyl transferase which confers resistance to glufosinate ammonium containing herbicides
- NOS terminator, derived from the nopaline synthase (*nos*) gene of *A. tumefaciens*

Besides these gene cassettes the *Not*I restriction fragment contains a 1.1 kb fragment of vector sequence upstream of the *cry1Ab* gene cassette. This fragment contains the ColE1 *ori*, the origin of replication that permits replication of plasmids in *Escherichia coli*, but which is not functional in plants.

Maize line Bt11 contains a single DNA insertion with one copy of the *Not*I restriction fragment.

Maize line MIR604:

The *mcry3A* and *pmi* gene cassettes were introduced in maize line MIR604 via Agrobacterium-mediated transformation.

The *mcry3A* gene cassette consists of the following elements:

- MTL promotor, derived from the *Z. mays* metallothionein-like gene; provides root-preferential expression
- *mcry3A* gene, from *B. thuringiensis* subsp. *tenebrionis* and optimized for expression in maize; confers resistance to certain coleopteran insects
- NOS, terminator sequence from the nopaline synthase gene of *A. tumefaciens*

The *pmi* gene cassette consists of the following elements:

- ZmUbiInt promotor, derived from the *Z. 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 *A. tumefaciens*

Maize line MIR604 contains a single DNA insertion with one copy of the *mcry3A* and *pmi* gene cassettes.

Maize line GA21:

GA21 maize was produced by microprojectile bombardment of *Z. mays* suspension cells using a *Not*I restriction fragment which contains the *mepsps* gene cassette.

The *Not*I restriction fragment contains the following elements:

- ract1 promoter, first intron and exon, derived from the rice actin 1 (*ract1*) gene
- optimized CTP, N-terminal chloroplast transit peptide (CTP) based on CTP sequences from sunflower and maize
- *mepsps* gene, modified 5-enolpyruvylshikimate-3-phosphate synthase gene from maize
- NOS terminator, derived from the nopaline synthase (*nos*) gene of *A. tumefaciens*

Maize line GA21 contains a single DNA insertion with six (partial) copies of the *Not*I restriction fragment.

Molecular analysis

The molecular characterization of maize lines Bt11, MIR604 and GA21 has been discussed in several previous advices (1-9). In these advices COGEM states that the molecular characterization does not give any reason to assume that these maize lines would pose a risk to the environment.

In the recent applications concerning import and processing of Bt11xGA21, Bt11xMIR604, MIR604xGA21 and Bt11 (renewal) the original sequence data of maize lines Bt11 and MIR604 was revised (2, 7, 8, 9). As mentioned in the previous advices concerning these applications, COGEM is of the opinion that both the original and the revised sequence data give no reason to expect any adverse effects on the environment (2, 8, 9).

Apparently the data presented in the first applications contained flaws. COGEM emphasizes that competent authorities depend on the information provided by the applicant. Therefore, COGEM underlines that these deviations are a cause of concern because a proper risk assessment can only be carried out if correct and clear-cut information is provided to the competent authorities.

Properties of the introduced genes conferring insect resistance

Maize line Bt11xMIR604xGA21 contains the *cry1Ab* and *mcry3A* genes. The *cry1Ab* gene encodes a δ-endotoxin specific for certain lepidopteran insects, e.g. the European corn borer (*Ostrinia nubilalis*) and the Mediterranean corn borer (*Sesamia nonagrioides*), whereas the *mcry3A* gene encodes a δ-endotoxin specific for certain coleopteran insects, e.g. the Western corn rootworm (*Diabrotica virgifera virgifera*) and the Nothern corn rootworm (*Diabrotica barberi*). The δ-endotoxins are solubilized in the midgut of susceptible insects and are activated by midgut proteases to release a toxin fragment. The

toxin fragment binds to specific receptors on the epithelial surface of the midgut. Subsequently, pores are formed in the membranes of the gut cells of the insect, enabling midgut bacteria to enter the body cavity, which leads to septicemia and death (11).

Properties of the introduced genes conferring herbicide tolerance

In addition to the *cry1Ab* and *mcry3A* genes, Bt11xMIR604xGA21 contains the *pat* and *mepsps* genes.

In non-transgenic plants glufosinate ammonium inhibits the activity of glutamine synthetase, an enzyme necessary for the production of glutamine and for ammonia detoxification. The application of glufosinate ammonium leads to reduced glutamine and increased ammonia levels in non-transgenic plants (12). Photosynthesis is inhibited and eventually the plant dies (13). In Bt11xMIR604xGA21 the PAT protein acetylates L-phosphinothricin, the active isomer of glufosinate ammonium. The resulting compound N-acetyl-L-phosphinothricin does not inhibit the activity of glutamine synthetase (12). As a result maize line Bt11xMIR604xGA21 is tolerant to L-phosphinothricin and thus to glufosinate ammonium containing herbicides.

In non-transgenic plants glyphosate inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme involved in the biosynthesis of aromatic amino acids. Application of glyphosate results in a lack of amino acids essential for growth and development of plants. Maize line Bt11xMIR604xGA21 expresses a modified EPSPS protein, which is not inhibited by glyphosate, and is therefore tolerant to glyphosate containing herbicides (13).

Properties of the introduced selection marker

Bt11xMIR604xGA21 also contains the *pmi* (*manA*) gene, which encodes the phosphomannose isomerase (PMI) enzyme. As a result maize plants are able to use 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.

The ability to use mannose as a carbon source is used to select transformed cells in cell cultures.

Environmental risk assessment

During the long process of domestication, maize has lost the ability to survive in the wild. In addition, maize needs human intervention to disseminate its seed. Maize kernels exhibit no dormancy and can only survive within a narrow range of climatic conditions. Furthermore, maize is very sensitive to weed competition and cannot persist as a weed (10, 15). In the Netherlands, volunteers are rarely found and establishment of maize

plants in the wild has never been observed. There are no reasons to assume that the introduced traits will increase the potential of Bt11xMIR604xGA21 to establish feral populations in case of incidental spillage.

General surveillance plan

General surveillance has been introduced to be able to observe unexpected effects of the cultivation of genetically modified crops on the environment. The setting or population in which these effects might occur is either not, or hardly predictable.

In the present application, a detailed general surveillance plan is provided to observe and register adverse effects of the import of Bt11xMIR604xGA21 timely. Following the initial placing on the market, the authorization holder will submit general surveillance reports on an annual basis for the duration of the authorization period.

Observations for unanticipated adverse effects will be monitored by existing systems which include the authorization holder and operators involved in the handling and use of viable Bt11xMIR604xGA21 maize. Operators involved in the import, handling and processing of Bt11xMIR604xGA21 maize inform the European trade associations (COCERAL, UNISTOCK and FEDIOL) of observed adverse effects. The trade associations report these effects to the authorization holder via the European Association of Bioindustries (EuropaBio) or directly to the authorization holder. EuropaBio is an association of members of the plant biotechnology industry which hosts a website containing information on approved genetically modified plants subject to general surveillance. The website contains an e-mail address and a telephone number to exchange information on the plants. COGEM points out that to gather general surveillance data a questionnaire would be helpful. By placing such a list on the website, essential information on adverse effects can be collected in a more coherent and consistent manner.

As mentioned in previous advices, COGEM prefers independent organizations which have expertise on the environment and whose activities in general surveillance continue after the authorization period, to be involved in general surveillance. In a previous advice on post-market monitoring, COGEM has outlined the standards that have to be met by a post-market monitoring system and has identified organizations which could be involved in post-market monitoring in the Netherlands (16).

Advice

COGEM has been asked to advice on import and processing for use in feed and food of maize line Bt11xMIR604xGA21.

Maize has lost the ability to survive in the wild. In addition, maize needs human intervention to disseminate its seed. In the Netherlands, volunteers are rare and establishment of maize plants in the wild has never been observed. There is no reason to assume that the expression of the *cry1Ab, mcry3A, pat, mepsps* and *pmi* genes in

Bt11xMIR604xGA21 increases the potential of maize to establish feral populations. In addition, introgression of the introduced genes into closely related species cannot occur, as wild relatives of maize are not present in Europe. In view of the above, COGEM is of the opinion that incidental spillage of maize line Bt11xMIR604xGA21 poses negligible risks to the environment. Therefore, COGEM considers the environmental risks associated with import and processing of maize line Bt11xMIR604xGA21 negligible.

Additional comment

COGEM abstains from advices on the potential risks of incidental consumption in case a food/feed assessment is already carried out by other organizations. This application is submitted under Regulation (EC) 1829/2003, therefore a food/feed assessment is carried out by EFSA. Other organizations who advice the competent authorities can perform an additional assessment on food safety although this is not obligatory. In the Netherlands a food and/or feed assessment for Regulation (EC) 1829/2003 applications is carried out by RIKILT and RIVM. Regarding the risks for food and feed, the outcome of the assessment by other organizations (EFSA, RIKILT, RIVM) was not known at the moment of the completion of this advice.

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