COMMISSIE
COGEM
GENETISCHE
MODIFICATIE

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KENMERK CGM/110823-01

ONDERWERP Advies: Import and processing of genetically modified maize line 5307

Geachte heer Atsma,

Naar aanleiding van een adviesvraag betreffende de import van de genetisch gemodificeerde maïslijn 5307 (EFSA/GMO/DE/2011/95) van Syngenta Crop Protection AG, deelt de COGEM u het volgende mee.

De COGEM is gevraagd om te adviseren over de mogelijke milieurisico's van import en verwerking van de genetisch gemodificeerde maïslijn 5307. Deze maïslijn brengt de genen *ecry3.1Ab* en *pmi* tot expressie. Als gevolg hiervan is maïslijn 5307 resistent voor bepaalde insecten uit de orde van de Coleoptera en in staat om mannose als koolstofbron te gebruiken. Deze laatste eigenschap is bedoeld voor de selectie van transgene planten in het productieproces. Maïslijn 5307 is nog niet eerder door de COGEM beoordeeld.
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 de transgene eiwitten het verwilderingspotentieel van Maïs vergroten. Bovendien zijn er in Europa geen wilde verwanten van Maïs aanwezig waardoor uitkruising niet mogelijk is. Gezien het bovenstaande acht de COGEM de kans dat incidenteel morsen tot verspreiding van deze maïslijn leidt verwaarloosbaar klein. Daarnaast is de COGEM van mening dat de moleculaire karakterisering adequaat is uitgevoerd.
Concluderend heeft de COGEM op basis van de door haar uitgevoerde risicobeoordeling geen bezwaar tegen import en verwerking van maïslijn 5307 en acht zij de risico's voor mens en milieu verwaarloosbaar klein. Omdat andere instanties een voedselveiligheidsbeoordeling uitvoeren, heeft de COGEM bij deze vergunningaanvraag de risico's van incidentele consumptie niet beoordeeld.

De door de COGEM gehanteerde overwegingen en het hieruit voortvloeiende advies treft u hierbij aan als bijlage.

Hoogachtend,

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Import and processing of genetically modified maize line 5307

COGEM advice CGM/110823-01

The present application by Syngenta Crop Protection AG (file EFSA/GMO/DE/2011/95) concerns import and processing for use in feed and food of the genetically modified maize line 5307. Cultivation is not part of this application.

Maize line 5307 expresses the ecry3.1Ab *gene conferring resistance to certain coleopteran insects. In addition, this maize line expresses the* pmi *gene which acts as a selectable marker enabling transformed plant cells to utilize mannose as a carbon source.*

The applicant showed by Southern blot analyses that one copy of the insert is integrated at a single integration locus in the genome of maize line 5307 and that the backbone of the plasmid used for transformation is absent in maize line 5307. Bioinformatic analysis of the junctions of the insert and the maize genomic DNA identified twelve open reading frames (ORFs) that are delimited by putative stop codons. These junction sequences were analyzed for similarity to known toxins or allergens. No similarities were found. The molecular characterization of maize line 5307 meets the criteria of COGEM.

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 introduced genes 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 the risks for humans and the environment associated with import and processing of maize line 5307 are negligible. A food/feed safety assessment is carried out by other organizations. Therefore, COGEM abstains from advice on the potential risks of incidental consumption.

Introduction

The present notification EFSA/GMO/DE/2011/95 by Syngenta Crop Protection AG concerns import and processing of the genetically modified maize line 5307. This maize line was produced by introduction of the *ecry3.1Ab* gene encoding the eCry3.1Ab protein. As a result maize line 5307 is resistant to certain coleopteran insects like *Diabrotica virgifera virgifera* (Western corn rootworm) and related *Diabrotica* species. In addition, this maize line expresses the pmi gene which acts as a selectable marker enabling transformed plant cells to utilize mannose as a carbon source. Maize line 5307 has not been previously assessed by COGEM.

Aspects of the crop

Maize (*Zea mays* L.) is a member of the grass family *Poaceae.* Maize is a highly domesticated crop, originating from Central America. Although pollinating insects visit maize plants and

therefore insect pollination cannot be completely excluded, maize is predominantly wind pollinated.[1,2] According to literature, pollen viability varies between 30 minutes and 9 days.[2,3,4] In Europe, no wild relatives of maize are present and therefore hybridization with other species cannot occur.

Throughout the world the appearance of volunteers is very rare. Seed kernels are the only survival structures of maize.[5] Due to the structure of the corn cob (ear on a stiff central cob enclosed in husks) natural dissemination of the kernels rarely occurs. Maize needs human intervention to disseminate its seed.[5] In addition, kernels exhibit poor dormancy resulting in a short persistence. Besides, maize can only survive within a narrow range of climatic conditions and, as maize is originally a subtropical crop, it is frost-sensitive.[6] Maize is very sensitive to weed competition.[7] During the long process of domestication, maize has lost the ability to survive in the wild.[5] Establishment of maize plants in the wild has never been observed in the Netherlands and COGEM is not aware of any reports of wild maize plants elsewhere in Europe.

Molecular characterization

The genetically modified maize line 5307 was produced by *Agrobacterium tumefaciens*-mediated transformation using vector pSYN12274. This vector contains the *ecry3.1Ab* and *pmi* gene expression cassettes.

The *ecry3.1Ab* expression cassette consists of the following elements:

- Intervening sequence with restriction sites for cloning.
- CMP promoter; *Yellow leaf curling virus* centrum promoter region which provides constitutive expression in maize.
- Intervening sequence with restriction sites for cloning.
- *ecry3.1Ab*; An engineered *cry* gene active against certain coleopteran insect (*Diabrotica*) species.
- Intervening sequence with restriction sites for cloning.
- NOS terminator; Terminator sequence from the nopaline synthase gene of *A. tumefaciens* which provides a polyadenylation site.

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

- Intervening sequence with restriction sites for cloning.
- ZmUbiInt promoter; Promoter region from the maize polyubiquitin gene which provides constitutive expression in monocots.
- Intervening sequence with restriction sites for cloning.
- *pmi*; *Escherichia coli* gene encoding the enzyme PMI which catalyzes the isomeration of mannose-6-phospate to fructose-6-phosphate.
- Intervening sequence with restriction sites for cloning.
- NOS terminator; Terminator sequence from the nopaline synthase gene of *A. tumefaciens* which provides a polyadenilation site.
- Intervening sequence with restriction sites for cloning.

Properties of the introduced genetic elements

The *ecry3.1Ab* gene encodes the eCry3.1Ab protein conferring resistance against certain coleopteran insects, including western corn rootworm (*D. virgifera virgifera*). The eCry3.1.Ab protein is based on mCry3A, a protein derived from the native Cry3A protein from *Bacillus thuringiensis* subsp. *tenebrionis*, and on the Cry1Ab protein from *B. thuringiensis* subsp. *kurstaki* HD-1.

eCry3.1Ab was synthesized by exchange of some of the variable regions of Cry3A (mCry3A) (active against coleopteran species) and Cry1Ab (active against lepidopteran species). This results in a Cry protein with strong bioactivity against the larvae of western corn rootworm, which is not susceptible to either Cry3A or Cry1Ab.[8] At the N-terminus of the eCry3.1Ab, 22 amino acids were introduced. The next 459 amino acids are identical to those of mCry3A followed by 172 amino acids of Cry1Ab at the C-terminus.

According to the applicant the mode of action of eCry3.1Ab, like that of most other Cry proteins, is highly specific to certain insects. It is stated that the eCry3.1Ab protein demonstrated no insecticidal activity among Coleoptera outside the family Chrysomelidae and no biological activity has been observed in tests of multiple other organisms, including lepidopteran insects.

Maize line 5307 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-transgenic maize plants 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.

Molecular analysis

Southern blot hybridizations with *ecry3.1Ab* -, *pmi*-, CMP promoter-, ZmUbiInt promoter- and with a NOS terminator-specific probe showed that one copy of the *ecry3.1Ab* gene, *pmi* gene, CMP promoter and ZmUbiInt promoter are present in maize line 5307 and two copies of the NOS terminator. The presence of two copies of the NOS terminator is to be expected since both expression cassettes contain this terminator. The absence of plasmid backbone was confirmed by hybridization analysis.

DNA sequence analyses demonstrated a one nucleotide change in the maize line 5307 insert 48 bp upstream of the CMP promoter in a non-coding region. Sequence analysis further indicated that some truncation occurred at the right border (RB) and the left border (LB) ends of the insert. The entire RB along with three non-coding base pairs is missing and eight bp of the LB were absent. According to the applicant neither the nucleotide change nor the deletions had any effect on the functionality of the insert.

The applicant sequenced 1000 base pairs of the 5' and 3' flanking regions. Bioinformatic analysis of these flanking regions revealed that the insert is located in a repetitive region of the maize genome. Bioinformatic analysis of the junctions of the insert and the maize genomic DNA

further identified twelve open reading frames that were delimited by putative stop codons. These sequences were analyzed by alignment searches in an allergen database (FARRP Allergen Online database, 2011) and a public database containing known toxins (NCBI Entrez® Protein Database, 2011). The results of these analyses demonstrated no sequence similarities between any known toxins or allergens and the twelve putative polypeptides.

In conclusion, COGEM is of the opinion that the molecular characterization of maize line 5307 has been adequately performed and meets the criteria laid down by COGEM.[9]

Environmental risk assessment

During the long process of domestication, maize has lost the ability to survive in the wild.[5] Maize needs human intervention to disseminate its seed.[5] Maize kernels exhibit poor dormancy resulting in a short persistence. Maize is very sensitive to weed competition and cannot persist as a weed.[7] Furthermore, maize is naturally frost sensitive and can only survive within a narrow range of climatic conditions. In the Netherlands, volunteers are rarely found and establishment of maize plants in the wild has never been observed. In Europe, no wild relatives of maize are present and therefore hybridization with other species cannot occur.[5] COGEM is not aware of any reports of wild maize plants elsewhere in Europe.

Maize line 5307 expresses the *ecry3.1Ab* gene and *pmi* genes. As a result, the maize line is resistant to certain coleopteran insects in the family *Chrysomelidae* and able to use mannose as a carbon source. The current application concerns import and processing. In case of spillage maize kernels may be released into the environment. Maize kernels can only survive within a narrow range of climatic conditions. The introduced traits do not increase the ability of maize kernels to survive in the environment. There is also no reason to assume that maize line 5307 has an increased potential to establish feral populations in case of incidental spillage.

Since 2008, COGEM abstains from advices on the potential risks of incidental consumption in case a food/feed safety assessment is already carried out by other organizations. This application is submitted under Regulation (EC) 1829/2003, therefore a food/feed safety 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 safety assessment for Regulation (EC) 1829/2003 applications is carried out by RIKILT. The outcome of the assessments by EFSA and RIKILT was not known at the moment of the completion of this advice.

General surveillance

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

The GS plan states that unanticipated adverse effects will be monitored by existing monitoring systems which include the authorization holder and operators involved in the handling and use of viable maize line 5307. Recently, COGEM formulated criteria which GS plans concerning Dutch

applications for import and cultivation of GM crops have to comply with.[10] COGEM concluded that the GS plans could be improved by a guarantee that operators will monitor for unanticipated effects. In the present GS plan on maize line 5307 the authorization holder states that the operators have agreed to provide information relevant to the monitoring of maize line 5307 to the authorization holder. More important, it is stated that the authorization holder will be able to give evidence that the operators collect this information. This is in line with the criteria laid down by COGEM.[10]

Advice

COGEM has been asked to advice on import and processing of maize line 5307. This maize line expresses the *ecry3.1Ab* gene conferring resistance to certain coleopteran insects. In addition, this maize line expresses the *pmi* gene which acts as a selectable marker enabling transformed plant cells to utilize mannose as a carbon source. The molecular characterization of maize line 5307 meets the criteria of COGEM.

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 expression of the *ecry3.1Ab* and *pmi* genes increase the potential of maize to establish feral populations in case of incidental spillage. 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 the risks for humans and the environment associated with import and processing of maize line 5307 are negligible. A food/feed safety assessment is carried out by other organizations. Therefore, COGEM abstains from advice on the potential risks of incidental consumption.

Additional remark

COGEM points out that the agronomic trials conducted by the applicant were very limited in scale. The applicant used a randomized complete block design consisting of two row plots, 5.3 meters long with approximately 68 plants per plot. No further detail on the lay-out of the experiments is provided. In the view of the COGEM this design is too small to measure yield or other characteristics accurately. Plants growing on the edge of a plot when bordered by a path are exposed to higher levels of sunlight, greater fluctuations in temperature and lower levels of humidity than those growing in the more sheltered interior which influences the characteristics of the plant. If no paths are part of the lay-out, the outer half of the two experimental rows are vulnerable to trampling by observators, which affects the trial. The possible presence of buffer zones is not mentioned.

Since this application only concerns import and processing, the trial design is of minor concern. However COGEM points out that for possible future applications for cultivation of this maize line, the applicant should use bigger plot sizes to prevent edge and neighbor effects.

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