Import and processing of maize MIR604xGA21

COGEM advice CGM/080521-02

Summary

The present application by Syngenta Seeds S.A.S. (file EFSA/GMO/UK/2007/48) concerns import and processing for use in feed and food of the genetically modified maize line MIR604xGA21. Cultivation is not part of this application.

Maize line MIR604xGA21 was obtained by conventional cross-breeding of the two parental lines. Previously, COGEM issued positive advices on import and processing of maize line MIR604. COGEM also advised on import and processing of maize line GA21. COGEM considered the ecological risks associated with the import and processing of maize line GA21 negligible.

The hybrid maize line MIR604xGA21 contains the mcry3A gene conferring resistance to certain coleopteran insects. In addition, this maize line contains the pmi gene which acts as a selectable marker enabling transformed plant cells to utilize mannose as a primary carbon source. Finally, MIR604xGA21 contains a mepsps gene, resulting in tolerance to glyphosate containing herbicides.

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 MIR604xGA21 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 MIR604xGA21 poses negligible risks to the environment. Therefore, COGEM considers the risks associated with import and processing of maize line MIR604xGA21 negligible. Additionally, COGEM questions some aspects of the provided general surveillance plan and gives some comments on the revised information regarding the molecular characterization.

Introduction

The present application by Syngenta Seeds S.A.S., file EFSA/GMO/UK/2007/48, concerns the import and processing of maize line MIR604xGA21 for use in feed and food. Maize line MIR604xGA21 was obtained by conventional cross-breeding of the two parental lines MIR604 and GA21. The hybrid maize line MIR604xGA21 contains the *mcry3A*, *pat* and *mepsps* genes, which are constitutively expressed. As a result MIR604xGA21 is resistant to certain coleopteran insects and tolerant to glyphosate containing herbicides.

Previous COGEM advices

In 2005, COGEM issued a positive advice on import and processing of maize line MIR604 (1). COGEM has also advised on maize line GA21 and concluded that the ecological risks associated with the import and processing of this maize line are negligible (2, 3).

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 (4). In Europe, hybridization with other species cannot occur as wild relatives of maize are not present in Europe (4). 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 MIR604xGA21 was produced by traditional cross-breeding of the two genetically modified parental maize lines MIR604 and GA21. The molecular characterization of these parental lines is discussed below.

Parental maize line MIR604:

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. In a previous advice an overview of the properties of the genes inserted was given (1)

- Active ingredient cassette
  - MTL promotor, derived from the *Z. 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 *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*

In 2005, the flanking sequences of MIR604 were analysed for the first time. Based on this information, COGEM considered the molecular analysis of the maize line adequate (1). The integration patterns of the introduced genes in the parental lines remained 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. It was sufficiently proven that no novel ORF's potentially encoding new proteins or fusion proteins were present.

After permission was granted, Syngenta provided additional information regarding the molecular analysis. A new analysis on the flanking regions was performed and some changes in DNA sequences in comparison with the first analysis were detected. The changes are: 2 base pairs in the 5' flanking sequence and 14 base pairs in the 3' flanking sequence. The exact cause of these deviations (technical/analytical flaws or occurrence of mutations) remains unclear to COGEM. Besides, due to unclear presentation of the data, it took the advisers of COGEM a needless amount of time to interpret the data. Therefore COGEM urges the applicant to present the data in a clear manner.

The applicant examined if the changes in base pairs resulted in the formation of new open reading frames (ORFs). It was shown that a putative open reading frame (ORF) of 258 bp is present that comprises the last 13 bp of the 3' *NOS* terminator continuing 175 bp into the genomic 3' flanking region. After comparison to the NCBI Entrez Protein Database, the applicant concluded that the amino acid sequence had no significant homology to any known or putative toxins or allergens.

The applicant defined an ORF as a region that initiates with an ATG codon and ends with any of the three stop codons TAA, TAG or TGA. COGEM notes that translation may initiate with other codons or that an ORF, which does not start with an ATG, becomes part of a longer ORF by the process of pre-mRNA splicing. Also potential ORFs shorter than 50 amino acids should be included in the analysis. Therefore, COGEM is of the opinion that also ORFs that could initiate with other start codons should be examined as well. Besides, only ORFs longer than 50 amino acids are analyzed.

However, recently COGEM abstains from advices on the potential risks of incidental consumption in case a food/feed assessment is already carried out by other organizations. If an application is submitted under Regulation (EC) 1829/2003 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. If an application is submitted under Directive 2001/18/EC a food/feed assessment is not carried out by EFSA and then COGEM advices on the potential risks of incidental consumption.

Since this application is submitted under Regulation (EC) 1829/2003 a food/feed assessment is carried out by EFSA, RIKILT and RIVM. Therefore, an analysis of the formation of putative new ORFs is not carried out by COGEM.

COGEM was surprised with the revised sequence data and underlines that these subsequent deviations are a cause of great concern. However, both sequence datasets (old and revised) of the maize lines MIR604 give no reasons to expect any potential environmental risks.

In view of the above, COGEM is of the opinion that the molecular analysis of hybrid maize line MIR604xGA21 does not indicate that import and processing of this line would pose a risk to the environment. 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 completion of this advice.

<u>Parental maize line GA21:</u>

GA21 maize was produced by particle bombardment of maize suspension cells using a *Not*I restriction fragment.

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

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

The molecular characterization of the maize line GA21 was discussed recently in a COGEM advice (7). In previous advices an overview of the properties of the genes inserted was given as well (2, 7). COGEM was of the opinion that the molecular analysis of hybrid maize line MIR604xGA21 did not indicate that import and processing of this line would pose a risk to the environment.

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 (4, 5). 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 MIR604xGA21 to establish feral populations in case of incidental spillage.

General surveillance plan

Several organizations representing trade organizations that import or use viable maize are mentioned in the general surveillance plan. According to the applicant these organizations are ‘well-placed’ to detect unanticipated effects on human health or the environment. However, information concerning their expertise in the environment is not given. In addition, it is unclear whether these organizations have agreed to cooperate in the general surveillance of MIR604xGA21. As stated in previous advices, COGEM is of the opinion that the applicant should ascertain that information on potential adverse effects is obtained. In addition, COGEM would prefer independent organizations which have expertise on the environment 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 (7).

Furthermore, according to the applicant indirect or delayed effects will be reported at the stage of re-evaluation or at the end of a given consent. As stated before, in COGEM’s opinion all observed effects, including indirect and delayed effects, should be reported annually.

Advice

COGEM has been asked to advice on import and processing for use in feed and food of hybrid maize line MIR604xGA21.

COGEM is of the opinion that incidental spillage of maize line poses negligible risks to the environment. 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 *mcry3A*, *pmi* and *mepsps* genes in MIR604xGA21 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.

Additional remark

The flaws in the original dossier are causes of great concern within COGEM. COGEM emphasizes that the information provided by the applicant is the foundation on which the European legislation regarding authorization of GMOs is based. The original sequence data of this hybrid maize line proved to be incorrect for the parental maize line MIR604. COGEM urges the applicant to provide adequate, correct and clear-cut information to the competent authorities. Only then a proper risk assessment can be carried out.

If the information provided by the applicant later turns out to be incorrect or when another application is submitted, this can seriously harm the confidence of the competent authorities assessing this information. Moreover, this also applies to the confidence of European citizens in (future) consumer products containing GMOs and indirectly in the confidence in the European and national governments as well as the industry involved.

References

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