Import and processing of genetically modified maize line Bt11x59122xMIR604x1507xGA21

COGEM advice CGM/120816-02

Introduction

The present application (file EFSA/GMO/DE/2011/99) concerns import and processing for use in feed and food of the genetically modified maize line Bt11x59122xMIR604x1507xGA21. Cultivation is not part of this application.

Maize line Bt11x59122xMIR604x1507xGA21 expresses the cry1Ab, cry1F, cry34Ab1, cry35Ab1, cry3A, pat, mepsps *and* pmi *genes. As a result, the maize line is resistant to certain lepidopteran and coleopteran insects, tolerant to glyphosate and glufosinate-ammonium containing herbicides, and is able to survive on a selection medium with mannose as sole carbon source.*

Maize line Bt11x59122xMIR604x1507xGA21 was produced by means of conventional breeding between the five genetically modified parental maize lines. Previously, COGEM issued positive opinions on the import and processing of all parental lines and various crosses between these lines.

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 incidental spillage of Bt11x59122xMIR604x1507xGA21 poses a negligible risk to the environment. Since 2008, COGEM abstains from giving advice on the potential risks of incidental consumption in case a food/feed assessment is already carried out by other organisations.

COGEM considers the environmental risks associated with import and processing of maize line Bt11x59122xMIR604x1507xGA21 to be negligible.

Introduction

The scope of the present application (EFSA/GMO/DE/2011/99) filed by Syngenta Crop Protection AG. concerns import and processing of maize line Bt11x59122xMIR604x1507xGA21. Maize line Bt11x59122xMIR604x1507xGA21 expresses the *cry1Ab, cry1F, cry34Ab1, cry35Ab1* and *cry3A* genes conferring resistance to certain lepidopteran and coleopteran insects. In addition, the *mepsps* and *pat* genes are expressed resulting in tolerance to glyphosate and glufosinate-ammonium containing herbicides. The selectable marker gene *pmi* allows the plant to use mannose as a sole carbon source.

Maize line Bt11x59122xMIR604x1507xGA21 was produced by conventional crossbreeding of five genetically modified parental maize lines. In the United States of America the individual genetically modified parental maize lines were authorised for use as food and/or feed in 1996

(Bt11 and GA21), 2001 (1507), 2004 (59122) and 2007 (MIR604),.[1] They were also authorised for cultivation in 1996 (Bt11), 1997 (GA21), 2001 (1507), 2004 (59122) and 2007 (MIR604).[1] In Europe, parental maize lines Bt11, 1507, GA21, 59122 and MIR604, were authorised for import and processing in respectively 1998, 2006, 2006, 2007 and 2009.[1]

Previous COGEM advice

COGEM advised positively on import and processing of five parental maize lines[2,3,4,5,6] and on import and processing of hybrid maize lines Bt11xGA21, Bt11xMIR604, MIR604xGA21, Bt11xMIR604xGA21 and 59122x1507.[7,8,9,10,11] COGEM also advised positively on cultivation of parental lines Bt11, 1507, GA21, and 59122[12,13,14,15] and hybrid line 1507x59122.[16]

Cultivation of MIR604 was also assessed by COGEM, but the data on non-target organisms (NTOs) was found insufficient to allow a conclusion that cultivation of MIR604 exerts negligible effects on NTOs.[17] In case of parental line 59122, COGEM was of the opinion that cultivation of this line poses a negligible risk to human health and the environment, under the condition that specific monitoring for ladybird beetles is incorporated.[18] Since the current application concerns import, previous remarks on NTOs are of minor importance.

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.[19,20] In Europe, no wild relatives of maize are present and therefore hybridisation with other species cannot occur.

Throughout the world the appearance of volunteers is very rare. Seed kernels are the only survival structures of maize.[21] 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.[21] 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.[22] Maize is very sensitive to weed competition.[23] During the long process of domestication, maize has lost the ability to survive in the wild.[21] 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 characterisation

Maize line Bt11x59122xMIR604x1507xGA21 was produced by crossing five genetically modified parental maize lines. COGEM previously concluded that the molecular characterisation of the parental maize lines was adequate.[2,3,4,5,6] Updated bioinformatic analyses of flanking sequences and putative polypeptides spanning the genomic DNA-insert junctions were provided for the parental lines Bt11, GA21 and MIR604. These analyses did not reveal any new concerns.

Properties of the introduced genes conferring insect resistance

Maize line Bt11x59122xMIR604x1507xGA21 contains the *cry1Ab, cry1F, cry34Ab1, cry35Ab1* and *cry3A* genes. Each of these genes encodes a different delta-endotoxin (δ-endotoxin), which target lepidopteran insects, such as the European corn borer *(Ostrinia nubilalis*), or coleopteran insects, such as corn rootworm larvae (*Diabrotica* spp.). Cry34Ab1 and Cry35Ab1 are referred to as a binary toxin pair, since they require each other for an effect on target insects.[24]

δ-endotoxins are solubilised in the midgut of susceptible insects and are activated by midgut proteases to release a toxin fragment. This toxin fragment binds to specific receptors on the epithelial surface of the midgut and causes pores to open. This leads to disruption of the movement of solutes across the gut epithelium and allows gut bacteria to escape the midgut and enter the hemolymph where they cause septicaemia and death.[25,26]

Properties of the introduced genes conferring herbicide tolerance

Maize line Bt11x59122xMIR604x1507xGA21 contains three copies of the *pat* gene encoding the enzyme phosphinothricin acetyltransferase protein (PAT). PAT confers tolerance to glufosinate-ammonium containing herbicides.[27] The active ingredient in glufosinate-ammonium is L-phosphinothricin (L-PPT), which binds to glutamine synthetase in plants. The detoxification of ammonia is thereby prevented, leading to plant death. The PAT enzyme catalyses the conversion of L-PPT to an inactive form, which does not bind glutamine synthetase. Therefore, the application of glufosinate-ammonium containing herbicides to maize line Bt11x59122xMIR604x1507xGA21 will not result in the build-up of ammonia and subsequent plant death.[28]

Bt11x59122xMIR604x1507xGA21 also expresses the *mepsps* gene. The *mepsps* gene encodes a modified 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) with two amino acid substitutions in comparison to the wildtype EPSPS. A chloroplast transit peptide sequence is fused to the *mepsps* gene, resulting in the transport of the mEPSPS protein to the chloroplast.[29]

EPSPS is a natural occurring enzyme involved in the biosynthesis of aromatic amino acids and is active in the chloroplasts of a plant cell. Glyphosate inhibits EPSPS, resulting in a lack of amino acids essential for growth and development of plants.[30,31] The mEPSPS enzyme has a decreased binding affinity of the protein for glyphosate, rendering the plant tolerant to application of glyphosate containing herbicides.

Properties of the introduced selectable marker

Bt11x59122xMIR604x1507xGA21 expresses the gene *pmi* (*manA*) encoding for the enzyme phosphomannose isomerase (PMI). As a result of the expression of *pmi*, plants are capable of using mannose as a sole carbon source.[32] Mannose is phosphorylated to mannose-6-phosphate (M6P) which can be converted to fructose-6-phosphate by the action 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 expression of *pmi* allows selection of GM plants. 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.

Environmental risk assessment

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. There is no reason to assume that maize line Bt11x59122xMIR604x1507xGA21 has an increased potential to survive or establish feral populations in case of incidental spillage.

Since 2008, COGEM abstains from giving advice on the potential risks of incidental consumption in case a food/feed assessment is already carried out by other organisations.[33] This application is submitted under Regulation (EC) 1829/2003, therefore a food/feed assessment is carried out by EFSA. Other organisations who advise 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. Regarding the risks for food and feed, the outcome of the assessment by other organisations (EFSA, RIKILT) was not known at the moment of the completion of this advice.

General surveillance plan

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 is required for every application for market authorisation. The current GS plan states that unanticipated adverse effects will be monitored by the authorisation holder and operators involved in the handling and use of viable maize Bt11x59122xMIR604x1507xGA21. The authorisation holder will also research possibilities for the involvement of existing monitoring networks.

In 2010, COGEM published a report on the principles that, according to COGEM, should be followed for general surveillance.[34] COGEM considers the widely used GS plan, accompanying the current application, adequate for import of Bt11x59122xMIR604x1507xGA21. However, the GS plan could be improved on the following points.

COGEM points out that adding a statement that the applicant will make raw data and analysis of monitoring data available to the Competent Authorities and the European Commission, could improve the GS plan.[35] This request is also made by EFSA in the guidance document.[36]

The GS plan states that if the authorisation holder identifies an unexpected adverse effect caused by the GM plant, he will inform the European Commission immediately. COGEM is of the opinion that Member States should also be directly informed of these effects by the authorisation holder, to ensure that appropriate measures for protection of humans and the environment can be implemented immediately.

Advice

COGEM has been asked to advise on import and processing of maize line Bt11x59122xMIR604x1507xGA21. In view of the above, COGEM is of the opinion that import

and processing of maize line Bt11x59122xMIR604x1507xGA21 poses a negligible risk to the environment.

References

1. GM crop database. http://cera-gmc.org/index.php?action=gm_crop_database (February 2012)
2. COGEM (2009). Renewal of authorization for import and processing of maize Bt11: additional information. Advies CGM/090310-01
3. COGEM (2003). Insect resistant and glufosinate ammonium tolerant transformation event 1507 maize. Advies CGM/030115-01
4. COGEM (2008). Toelichting op eerder COGEM advies over import en verwerking van mais GA21. Advies CGM/080117-02
5. COGEM (2005). Import and processing of maize variety 59122. Advies CGM/ 051122-01
6. COGEM (2005). Import and processing of maize variety MIR604. Advies CGM/051122-02
7. COGEM (2009). Import and processing of BT11xGA21. Advies CGM/091019-02
8. COGEM (2010). Additional advice on import and processing of genetically modified maize Bt11xMIR604. Advies CGM/100608-02
9. COGEM (2010). Additional advice on import and processing of genetically modified maize MIR604xGA21. Advies CGM/100608-01
10. COGEM (2008). Import and processing of maize Bt11xMIR604xGA21. Advies CGM/081017-03
11. COGEM (2007). Import of genetically modified maize 59122 x 1507. Advies CGM/070911-02
12. COGEM (2003). Insect resistent and glufosinate ammonium tolerant transformation event 1507 maize. Advies CGM/030919-04
13. COGEM (2005). Assessment of an EFSA opinion on the cultivation of Bt11 maize. Advies CGM/050816-01
14. COGEM (2012). Aanvullend advies teelt GA21 n.a.v. verschijnen EFSA opinie. Advies CGM/120124-01.
15. COGEM (2008). Cultivation of genetically modified maize line 59122. Advies CGM/080207-02
16. COGEM (2008). Cultivation of genetically modified maize line 1507x59122. Advies CGM/080325-02
17. COGEM (2011). Teelt insectenresistente maïslijn MIR604. Advies CGM/110404-01
18. COGEM (2008). Cultivation of genetically modified maize line 59122. Advies CGM/080207-02
19. Hin CJA (2001). Landbouwkundige risico's van uitkruising van GGO-gewassen. Centrum voor Landbouw en Milieu (CLM)
20. Treau R & Emberlin J (2000). Pollen dispersal in the crops Maize (*Zea mays*), Oil seed rape (*Brassica napus* ssp. *oleifera*), Potatoes (*Solanum tuberosum*), Sugar beet (*Beta vulgaris* ssp. *vulgaris*) and Wheat (*Triticum aestivum*)- Evidence from publications. Soil Association
21. OECD (2003). Consensus document on the biology of *Zea mays* subsp. *mays* (Maize)
22. Miedema P (1982). The effects of low temperature on *Zea mays*. Advances in Agronomy 35: 93-128

23. Crop Protection Compendium (2007). *Zea mays* (maize). CD-ROM edition, © Cab International 2007, Nosworthy way, Wallingford, UK
24. Schnepf HE *et al.* (2005). Characterization of Cry34/Cry35 Binary Insecticidal Proteins from Diverse *Bacillus thuringiensis* Strain Collections. Appl Environ Microbiol. 2005 April; 71(4): 1765–1774.
25. Sanahuja *et al.* (2011). Bacillus thuringiensis: a century of research, development and commercial applications. Plant Biotechnol J. 9: 283-300
26. Broderick NA *et al.* (2006). Midgut bacteria required for *Bacillus thuringiensis* insecticidal activity. Proc. Natl. Acad. Sci. USA. 103: 15196-15199
27. Manderscheid R & Wild A. (1986). Studies on the mechanism of inhibition by phosphinothricin of glutamine synthetase isolated from Triticum aestivum L. J. Plant Physiol. 123: 135-142
28. Strauch E *et al.* (1988). Cloning of a phosphinothricin Nacetyltransferase gene from Streptomyces viridochromogenes Tü494 and its expression in Streptomyces lividans and Escherichia coli. Gene 63: 65-74
29. Della-Cioppa GS *et al*. (1986). Translocation of the precursor of 5-enolpyruvylshikimate-3-phosphate synthase into chloroplasts of higher plants in vitro. Proceedings of the National Academy of Sciences 83:6873-6877
30. Green JM (2007). Review of glyphosate and ALS-inhibiting herbicide crop resistance and resistant weed management. Weed technology 21: 47-558
31. Funke T *et al*. (2006). Molecular basis for the herbicide resistance of Roundup Ready crops. Proceedings of the National Academy of Sciences of the United States of America: 103:13010-13015
32. Joersbo M & Okkels FT (1996). A novel principle for selection of transgenic plant cells: positive selection. Plant Cell Reports 16, 219-221
33. COGEM (2008). Toelichting advies GA21. Brief CGM/080117-02
34. COGEM (2010). General Surveillance. Signalering CGM/100226-01
35. COGEM (2011). Advies m.b.t het concept van de herziene 'Guidance on the Post-Market Environmental Monitoring (PMEM) of GM plants' van de EFSA. Advies CGM/110520-01
36. EFSA Panel on Genetically Modified Organisms (2011). Guidance on the Post-Market Environmental Monitoring (PMEM) of genetically modified plants. EFSA Journal 9:2316