

Import and processing of genetically modified maize line Bt11xMIR162x1507xGA21

COGEM advice CGM/120816-01

Introduction

The present application (file EFSA/GMO/DE/2010/86) concerns import and processing for use in feed and food of the genetically modified maize line Bt11xMIR162x1507xGA21. Cultivation is not part of this application.

Maize line Bt11xMIR162x1507xGA21 expresses the cry1Ab, cry1F, vip3Aa20, pat, mepsps and pmi genes. As a result, the maize line is resistant to certain lepidopteran 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 Bt11xMIR162x1507xGA21 was produced by means of conventional breeding between the four genetically modified parental maize lines. Previously, COGEM issued positive opinions on the cultivation, import and processing of the parental lines and on two 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 Bt11xMIR162x1507xGA21 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 Bt11xMIR162x1507xGA21 to be negligible.

Introduction

The scope of the present application (EFSA/GMO/DE/2010/86) filed by Syngenta Crop Protection AG. concerns import and processing of maize line Bt11xMIR162x1507xGA21. This maize line expresses the *cry1Ab*, *cry1F* and *vip3Aa20* genes conferring resistance to certain lepidopteran 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 Bt11xMIR162x1507xGA21 was produced by conventional crossbreeding of four 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) and 2008 (MIR162).¹ They were also authorised for cultivation in 1996 (Bt11), 1997 (GA21), 2001 (1507) and 2010 (MIR162).¹ In Europe, parental maize lines

Bt11, 1507 and GA21 were authorised for import and processing in respectively 1998, 2006, and 2006.¹ EFSA has recently concluded that MIR162 is as safe as its conventional counterpart concerning import and finalised its overall opinion for authorisation.

Previous COGEM advice

COGEM advised positively on import and processing of four parental maize lines^{2,3,4,5} and on import and processing of hybrid maize lines Bt11xGA21 en Bt11xMIR162xGA21.^{6,7} COGEM also advised positively on cultivation of parental lines Bt11, 1507 and GA21.^{8,9,10}

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.^{11,12,13} 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.¹⁴ 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.¹⁴ 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.¹⁵ Maize is very sensitive to weed competition.¹⁶ During the long process of domestication, maize has lost the ability to survive in the wild.¹⁴ 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 Bt11xMIR162x1507xGA21 was produced by crossing four genetically modified parental maize lines. COGEM previously concluded that the molecular characterisation of the parental maize lines was adequate.^{2,3,4,5} Updated bioinformatic analyses of flanking sequences and putative polypeptides spanning the genomic DNA-insert junctions were provided for parental lines Bt11, GA21 and MIR162. These analyses did not reveal any new concerns.

Properties of the introduced genes conferring insect resistance

Maize line Bt11xMIR162x1507xGA21 contains the *cry1Ab*, *cry1F* and *vip3Aa20* genes. Each of the *cry* genes encodes a different delta-endotoxin (δ -endotoxin), which target lepidopteran insects such as the European corn borer (*Ostrinia nubilalis*). The Vip3A protein also targets lepidopteran insects, by a mechanism highly similar to δ -endotoxins.¹⁷

δ -endotoxins and Vip proteins 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. Vip proteins bind to different receptors than δ -endotoxins, but both cause 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.^{18,19}

Properties of the introduced genes conferring herbicide tolerance

Maize line Bt11xMIR162x1507xGA21 contains two copies of the *pat* gene encoding the enzyme phosphinothricin acetyltransferase protein (PAT). PAT confers tolerance to glufosinate-ammonium containing herbicides.²⁰ 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 Bt11xMIR162x1507xGA21 will not result in the build-up of ammonia and subsequent plant death.²¹

Bt11xMIR162x1507xGA21 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.²²

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.^{23,24} 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

Bt11xMIR162x1507xGA21 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.²⁵ 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 insertion of *pmi* has led to the introduction of a selection system. 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 Bt11xMIR162x1507xGA21 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.²⁶ 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 Bt11xMIR162x1507xGA21. 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.²⁷ COGEM considers the widely used GS plan, accompanying the current application, adequate for import of Bt11xMIR162x1507xGA21. 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.²⁸ This request is also made by EFSA in the guidance document.²⁹

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 Bt11xMIR162x1507xGA21. In view of the above, COGEM is of the opinion that import and processing of maize line Bt11xMIR162x1507xGA21 poses a negligible risk to the environment.

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