Import of genetically modified maize line MON88017

COGEM advice CGM/070308-01

This notification concerns the import and processing of the genetically modified maize line MON88017 for use in feed and food. The maize line harbors the genes cp4epsps and cry3Bb1. Cp4epsps confers tolerance to herbicides containing the active ingredient glyphosate. The cry3Bb1 gene is responsible for resistance to certain coleopteran insects such as the corn rootworm (Diabrotica virgifera). Cultivation is not part of the application.

Previously, COGEM advised positively on the import of maize line MON863 which expresses the cry3Bb1 gene. COGEM has also given a positive advise on the hybrid maize line MON863 x NK603 which expresses a cp4epsps gene besides the aforementioned cry3Bb1 gene. Maize line MON863 and MON863 x NK603 are already commercially grown in the U.S.A.

In the Netherlands, no wild relatives of maize are present and establishment of maize plants in the wild has never been observed. There are no reasons to assume that the inserted traits will increase the potential of the maize line to establish feral populations. In addition, the appearance of volunteers is very rare under Dutch conditions.

The experts of the COGEM are of the opinion that interactions between recombinant gene products are not expected. The applicant has sufficiently proven that no toxic or allergenic products are formed as a result of the modification of maize line MON88017. Based on these considerations, COGEM is of the opinion that the import of maize line MON88017 poses a negligible risk to human health and the environment.

Introduction

The scope of the present notification by Monsanto Company concerns the import and processing of maize line MON88017 for use in feed and food.

The maize line is modified by the introduction and expression of the genes *cp4epsps* and *cry3Bb1* conferring tolerance to glyphosate containing herbicides and insect resistance to certain *coleopteran* insects such as the corn rootworm *(Diabrotica virgifera).*

Previous COGEM advices

In the past, COGEM advised positively on the import and processing of MON863 for use in feed and food which expresses the *cry3Bb1* gene and is therefore less susceptible for certain coleopteran insects (11). COGEM has also advised positively on the import of the hybrid maize line MON863 x NK603 (12). The parental lines contain the genes *cry3Bb1*

and *cp4epsps*, conferring resistance to certain coleopteran insects, and tolerance to herbicides containing the active ingredient glyphosate, respectively.

Furthermore, COGEM has advised positively on the import and cultivation of genetically modified maize line NK603 expressing the *cp4epsps* gene (13) (14). Maize line MON863 and MON863 x NK603 are already commercially grown in the U.S.A.

The current application concerns the import and processing of maize line MON88017 containing the genes *cp4epsps* and *cry3Bb1*. In contrast to former applications, MON88017 is not created by crossing of two parental maize lines but by transformation. Another difference with former applications can be found in the Cry3Bb1 protein, which differs by one amino acid from earlier mentioned Cry3Bb1 proteins.

Maize line MON88017

Aspects of the crop

Maize (*Zea mays L.*) is a member of the grass family *Poaceae.* Maize is being cultivated as an agricultural crop, originating from Central America. Although insect pollination can not 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 can not occur.

The appearance of volunteers is very rare under Dutch conditions. Grains exhibit no germination dormacy, resulting in a short persistence. In addition, only few seeds remain on the field after harvesting of fodder maize (1). Establishment of maize plants in the wild has never been observed in the Netherlands.

Molecular characterization

The genetically modified maize line MON88017 was produced by Agrobacterium tumefaciens-mediated transformation.

An overview of the introduced sequences is given below:

- Cry3Bb1 expression cassette:
  - P-e35S, originating from *Cauliflower mosaic virus*, promoter with a duplicated enhancer region
  - wt CAB, originated from *Triticum aestivum* (wheat); 5' untranslated leader of the wheat chlorophyll a/b binding protein
  - ract1 intron, derived from *Oryza sativa* (rice); promotes transcription

- MON88017 *cry3Bb1*, originating from *Bacillus thuringiensis subsp. Kumamotoensis*, DNA sequence coding for a genetic variant of CryBb1 protein.
- Tahsp 17 3', wheat heat shock protein derived from *T. aestivum*; stops transcription and induces the polyadenylation

- cp4epsps expression cassette:
  - P-ract1/ ract1 intron, promoter and intron derived from *O. sativa*, intron promotes transcription
  - *Ctp2* gene from *Arabidopsis thaliana*; encoding the N-terminal chloroplast transit peptide
  - *cp4epsps* gene from *A. tumefaciens* CP4; encoding 5-enolpyruvlshikimate-3-phosphatesynthase (epsps)
  - NOS 3', terminator from *A. tumefaciens*; terminates transcription

Properties of the introduced genes conferring herbicide tolerance

Cp4epsps encodes Cp4epsps proteins possessing a high tolerance to glyphosate. With the introduction of *cp4epsps* in maize MON88017 tolerance was obtained to glyphosate containing herbicides.

Epsps is a natural occurring enzyme involved in the biosynthesis of aromatic amino acids. In non-transgenic maize lines, glyphosate acts by binding to, and thus inhibiting the function of naturally occurring epsps. Consequently, aromatic amino acids are no longer formed, leading to plant death. In contrast, Cp4epsps is not affected by glyphosate because of a reduced binding affinity. MON88017 expresses the gene *cp4epsps*, resulting in a high tolerance to glyphosate. The application of this herbicide will therefore not cause death of maize line MON88017, because the plant is still able to produce aromatic amino acids (5).

Epsps proteins are active in the chloroplasts of a plant cell. The *ctp2* gene is fused to the epsps transgene, resulting in the transport of the transgenic Epsps protein to the chloroplast (7).

Properties of the introduced genes conferring insect resistence

MON88017 was genetically modified with the *cry3Bb1* gene derived from *B. thuringiensis* (subsp. Kumamotoensis). The produced Cry3Bb1, a δ-endotoxin, is lethal to certain insects of the coleopteran order, including larvae of the corn rootworm (*Diabrotica sp.).* The δ-endotoxin selectively binds to receptors located in the midgut of susceptible insects (6). Following binding, the gut is perforated enabling enterobacteria from the midgut to enter the body, causing the insect to die from poisoning within 48 to 120 hours (10).

The corn rootworm is an economically important pest insect, which causes major crop losses. Larvae of this insect feed on maize roots, resulting in the interference of the plant's ability to absorb water and nutrients and in the reduction of the stability of the plant. As a consequence, damaged plants may lodge, making harvesting difficult.

Corn rootworm was accidentally introduced in the mid-nineties in Bosnia presumably by military air traffic and became established shortly after introduction. The pest is still spreading at regular rate of about 40 km per year, but is infamous for rapid spread over large distances by (air) traffic. In 2003 this insect was first discovered near the airport of Schiphol in the Netherlands, but eradicated successfully. If the corn rootworm is able to establish and spread itself in the Netherlands, large damage to crops can be expected (8, 9).

The Cry3Bb1 protein does not exhibit amino acid homology or protein structure homology to known allergens or toxins.

Molecular analysis

To the opinion of the experts of the COGEM, interactions between recombinant gene products are not expected in the maize line because the gene products accumulate in different cellular compartments. The protein Cry3Bb1 accumulates in the cytoplasm, while Cp4epsps is directed to the chloroplasts.

Furthermore, the genes and their gene products have a history of safe use. Products and derivatives of the maize lines expressing these genes have been handled and consumed without reports of side-effects.

Respectively 878 nucleotides on the 5' and 1000 nucleotides on the 3' flanking sequences of the insert were analysed and confirmed to be genomic Maize DNA. The applicant performed extensive bioinformatic analysis. Amongst others the six reading frames were analysed for the presence of ORF's spanning the junction of the insert and the genomic DNA. All putative proteins encoded by the identified putative ORF's were compared with known sequences in databases. None of them showed homology with known toxins and allergens.

Therefore, COGEM is of the opinion that the applicant has sufficiently proven that the Cry3Bb1 protein in MON88017 poses a negligible risk to human health and the environment.

Advice

The present application concerns the commercial import and processing of maize line MON88017 for the use in food and feed. MON88017 expresses the genes *cp4epsps* and *cry3Bb1*, providing the plant with a herbicide tolerance trait as well as resistance to

certain coleopteran insects. In the past, COGEM advised positively on maize lines with these traits and hybrid maize lines with both traits.

There are no wild relatives of maize in the Netherlands and the appearance of volunteers is very rare under Dutch conditions. Furthermore, there are no reasons to assume that the inserted traits will increase the now absent potential of the maize line to run wild.

In the opinion of COGEM, the molecular analysis is adequate and it is sufficiently proven that the putative novel proteins encoded by the identified putative ORF's do not share homology with known allergens or toxins. Based on the data concerning feeding study experiments provided by the applicant, COGEM agrees that no adverse effects are likely to occur in case of consumption of MON88017 or products that contain MON88017. COGEM concludes that it was sufficiently proven that no toxic or allergenic products are formed as a result of the modification

In addition maize line MON863 and MON863 x NK603 are already commercially grown in the U.S.A. The inserted genes and gene products possess a history of safe use and no interactions between recombinant gene products are expected in the modified maize line MON88017.

In view of these considerations, COGEM is of the opinion that the proposed import and processing of maize line MON88017 poses a negligible risk to human health and the environment.

References

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