Cultivation of genetically modified maize line MON88017

COGEM advice CGM/081112-02

This notification concerns the cultivation of genetically modified maize line MON88017. 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). Previously, COGEM advised positively on the import of maize line MON88017.

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.

COGEM is of the opinion that the molecular characterization of MON88017 is adequate and complete. The applicant conducted several laboratory experiments to assess the effect of cry3Bb1 proteins expressed by maize MON88017 on non-target organisms. However, only one of these tests is conducted with the exact protein expressed in MON88017. COGEM is of the opinion that this is insufficient to conclude there are no adverse effects to be expected on NTOs with the cultivation of MON88017.

Furthermore, COGEM is of the opinion that the general surveillance plan supplied for cultivation of maize MON88017 is at some points too informal and gives no guarantees that sufficient data are collected. The applicant should describe in more detail how the general surveillance plan will be organized and which organizations are involved.
Based on the considerations put forward in this advice, COGEM cannot advise positively on the cultivation of maize line MON88017. COGEM is of the opinion that additional data from laboratory experiments and field studies have to be provided conducted with maize line MON88017 and with a Cry3bb1 variant as expressed in this maize line.

Introduction

The scope of the present notification (EFSA/GMO/CZ/2008/54) by Monsanto Company, as represented by Monsanto Europe S.A./N.V. concerns the cultivation of maize line MON88017. 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 2007, COGEM advised positively on the import and processing of genetically modified maize line MON88017 (11). COGEM concluded that there is no reason to assume that the traits introduced will increase the potential of maize line MON88017 to establish feral populations in case of incidental spillage. To obtain a permission to cultivate a genetically modified plant, additional data is required. Amongst others, the applicant has to provide evidence that the GM plant exerts no adverse effects on non-target organisms.

Aspects of the crop

Maize (*Zea mays L.*) is a member of the grass family *Poaceae.* Maize is 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 cannot 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/ I-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). This cry3Bb1 coding sequence was modified to encode six specific amino acid substitutions. The same amino acid substitutions are present in the cry3Bb1 gene that is expressed in genetically modified maize line MON863. MON863 has been cultivated in Canada and the USA and has a history of safe use. The cry3Bb1 gene in MON88017 differs by one amino acid (Apragine (D) instead of Glycine (G)) from cry3Bb1 in MON863. The applicant does not demonstrate possible consequences of this substitution.

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-endotoxins are solubilized in the midgut of susceptible insects and are activated by midgut proteases to release a toxin fragment. The toxin fragment binds to specific receptors on the epithelial surface of the midgut. Subsequently, pores are formed in the membranes of the gut cells

of the insect, enabling the content of the midgut and its bacteria to enter the body cavity, which leads to septicemia and death (6, 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 a regular rate of about 40 km per year, but is infamous for it's 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 in the Netherlands, extensive damage to crops can be expected (8, 9).

Molecular analysis

COGEM is of the opinion that interactions between recombinant gene products are not expected in maize line MON88017 because the gene products accumulate in different cellular compartments. The protein Cry3Bb1 accumulates in the cytoplasm, while Cp4epsps is directed to the chloroplasts (7). Furthermore, the genes inserted 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.
The presence of only one copy of the transformation cassette at a single locus of DNA integration was confirmed by Southern blot analysis. Moreover, no plasmid backbone sequences are present in the genome of maize line MON88017.

Respectively 878 nucleotides on the 5' and 1000 nucleotides on the 3' flanking sequences of the insert were analyzed and confirmed to be genomic maize DNA. The applicant performed an extensive bioinformatic analysis. Amongst others the six reading frames were analyzed 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. The applicant updated this part of the bioinformatics analysis compared to the import application that was issued in 2005 (EFSA/GMO/CZ/2005/27). The putative ORFs spanning the junction of the insert and the genomic DNA did not show any homology with known allergens or toxins. Furthermore, the Cry3Bb1 protein does not exhibit amino acid homology or protein structure homology to known allergens or toxins.

COGEM is of the opinion that the molecular analysis is performed adequately and concludes that the applicant has sufficiently proven that no toxic or allergenic products are formed as a result of the modification of maize line MON88017.

Environmental risk assessment

In the opinion of COGEM, there is no reason to assume that the traits introduced in maize MON88017 will increase the potential of maize to establish feral populations. With regard to potential adverse effects of MON88017 on non-target organisms (NTOs), the applicant refers to laboratory, greenhouse and field studies. These studies will be discussed below.

Laboratory studies

The applicant conducted several laboratory experiments to assess the effect of cry3Bb1 proteins expressed by maize MON88017 on non-target organisms. However, only one of these tests is conducted with the exact protein expressed in MON88017. Most laboratory tests are performed with variants of Cry3Bb1 proteins or with different variants of the *cry3Bb1* gene expressed in maize (e.g. MON863). The different variants were created by the applicant to discover the optimal expression of the Cry3Bb1 protein against the target pest. In the laboratory assays potential effects of cry3Bb1 protein (not the one from MON88017) or pollen are tested on a range of non-target organisms like earth worm, carabid beetle, ladybird beetle, monarch butterfly, honey bee, parasitic wasps, green lacewing, minute pirate bugs and Collembola.

The applicant is of the opinion, but does not provide evidence for this, that the Cry3Bb1 protein variants used in these tests have a similar functional and biological activity. The amino acid sequence of the Cry3Bb1 produced in MON88017 is >99.8% identical (one amino acid difference) to that of the Cry3Bb1 produced in MON863. Therefore, the applicant concludes that the activity of the two Cry3Bb1 proteins against the target insect species would be similar. The Cry3Bb1 protein variants expressed in the other maize lines used for laboratory experiments share an amino acid identity of >98% with MON88017 and MON863. The applicant concludes that the studies conducted with these other maize lines are considered appropriate to support the NTO risk assessment of MON88017.

Although some of the laboratory tests are conducted adequately, COGEM is of the opinion that the application of a similar but not identical Cry3Bb1 protein in these studies is a serious demerit. Furthermore, COGEM noted differences between the studies in the applied doses. One study identified 8000 ppm as 20 x natural doses while another mentions 1860 ppm as 20 x natural doses.

Field studies

The applicant provided a field study with maize line MON88017 performed in Germany (four locations) and Spain (four locations). In this field study the disease susceptibility to *Sesamia* spp and *Ostrinia nubilalis* was tested qualitatively by a categorical rating. These

qualitative data were not subjected to statistical analysis. Based on the results of this study, the applicant concluded that no effects on non-target insects were demonstrated. COGEM is of the opinion that quantitative data and not qualitative data are necessary to draw conclusions on the occurrence of effects on non-target organisms.

Two additional studies on MON88017 investigating the effect of MON88017 pollen on adult green lace wings and a field study in Germany to assess non-target effects on the rice leaf bug were recently published, However, these studies are not part of the dossier and do not adequately address effects on NTO's. The adult green lacewing (*Chrysoperla carnea*) belongs to the order of the Neuroptera and the rice leaf bug (*Trigonotylus caelestialium*) to the order of the Heteroptera. COGEM is of the opinion that studies on possible adverse effects on NTOs should at least include species of the order to which the Bt toxin is targeted e.g. the Coleoptera. The applicant did not provide results of such studies conducted with MON88017. Field studies on non-target effects on soil- foliage- and ground dwelling arthropods and mites were performed with maize line MON863 (expressing a cry3Bb1 protein that differs by one amino acid from MON88017) during field trials in the USA. The applicant concluded that no adverse effects were demonstrated.

COGEM cannot agree with the applicant that the cry3Bb1 variants used in the laboratory tests have a sufficient similar functional and biological activity to support the NTO risk assessment of MON88017. COGEM is of the opinion that sufficient evidence to support this conclusion is lacking and underlines the importance of a specific safety assessment regarding possible effects on (relevant) non-target organisms. Consequently, she is of the opinion that the studies provided are insufficient to conclude there are no adverse effects to be expected on NTOs with the cultivation of MON88017.

<u>General surveilance</u>

COGEM is of the opinion that the general surveillance plan supplied for cultivation of maize MON88017 is too general, lumping birds, deer and insects into one category "wildlife" and notices that questions need to be phrased differently to acquire data that can be used to detect negative or positive trends in populations of organisms relevant to the monitoring scheme. Additionally, the general surveillance plan relies mostly on the expertise of the users of this maize line. In her previous advice COGEM stated that in her opinion this is too limited to ascertain that unanticipated adverse effects are observed and reported. COGEM suggested to include existing monitoring systems in general surveillance. The Netherlands is in the process of implementing such a system. COGEM underlines the importance of using existing monitoring systems in other countries in general surveillance and including these monitoring systems in general surveillance. In

addition, COGEM is of the opinion that the general surveillance plan should mention that information about the distribution of the form and the percentage of returned forms is included in the monitoring report. 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

The present application concerns the cultivation of maize line MON88017. This genetically modified maize line expresses the *cp4epsps* and *cry3Bb1* genes, providing the plant with a herbicide tolerance trait as well as resistance to certain coleopteran insects. In the past, COGEM advised positively on import and processing of this maize line.

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 performed adequately and completely. COGEM concludes that it was sufficiently proven that no toxic or allergenic products are formed as a result of the modification of maize line MON88017. In addition maize line MON863 and MON863 x NK603 which harbors the genes *Cry3Bb1* and *Cry3Bb1* and *cp4epsps* are already commercially grown in the USA. 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.

The applicant conducted several laboratory and field studies to assess possible adverse effects of MON88017 on non-target organisms. However, only one of these tests is conducted with the exact protein expressed in MON88017. Most laboratory tests are performed with variants of Cry3Bb1 proteins or with different variants of the *cry3Bb1* gene expressed in maize (e.g. MON863). COGEM also places a few remarks on the quality of some of the studies provided and the selection of NTO's used in the experiments. Consequently, she is of the opinion that this is insufficient to conclude there are no adverse effects to be expected on NTOs with the cultivation of MON88017.

COGEM also places a remark on the general surveillance plan. The general surveillance plan supplied for cultivation of maize MON88017 is too informal at some points and gives no guarantees that sufficient data is obtained.

Conclusion

Based on the considerations put forward in this advice, COGEM is of the opinion that she can not perform a reliable risk analysis with regard to the cultivation of this maize

variety. As a result of the concerns mentioned regarding the NTO studies and the general surveillance plan, at this point COGEM cannot issue a positive advice on the cultivation of maize line MON88017.

COGEM is of the opinion that additional data from laboratory experiments and field studies have to be supplied conducted with maize line MON88017 and with a Cry3bb1 variant as expressed in this maize line. Furthermore, studies on possible adverse effects on NTOs should at least include species of the order to which the Bt toxin is targeted e.g. the Coleoptera.

References

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