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Aan de minister van
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Ordening en Milieubeheer
Mevrouw dr. J.M. Cramer
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DATUM 22 oktober 2007
KENMERK CGM/071022-02
ONDERWERP Advies import en verwerking van maïs MON89034 (EFSA/GMO/NL/2007/37)

Geachte mevrouw Cramer,

Naar aanleiding van de adviesvraag betreffende het dossier EFSA/GMO/NL/2007/37, getiteld “Import and processing of maize MON89034” voor de import en verwerking van genetisch gemodificeerde maïs door Monsanto S.A. adviseert de COGEM als volgt.

**Samenvatting:**
De COGEM is gevraagd te adviseren over toelating voor import en verwerking van de genetisch gemodificeerde maïslijn MON89034. De betreffende maïslijn bevat de *cry1A.105* en *cry2Ab2* genen waardoor de plant resistent is voor bepaalde insecten uit de orde van de Lepidoptera.
Verwildering van maïsplanten is in Nederland nooit waargenomen. Daarnaast is opslag van maïsplanten in Nederland nagenoeg uitgesloten. Er zijn geen redenen om aan te nemen dat expressie van de geïnserteerde genen het verwilderingspotentieel van maïs vergroten. Bovendien zijn er in Europa geen wilde verwanten van maïs aanwezig waardoor uitkruising niet mogelijk is. Daarom acht de COGEM de kans dat incidenteel morsen leidt tot verspreiding van MON89034 zeer klein.
De COGEM is echter van mening dat de gegevens betreffende de moleculaire karakterisering van MON89034 niet volledig zijn.
Bovendien wijst de COGEM erop dat het algemene monitoringsplan onvoldoende in detail is uitgewerkt waardoor het onduidelijk is of eventuele onverwachte effecten effectief gerapporteerd zullen worden.
De COGEM acht de kans zeer klein dat import en verwerking van deze maïslijn tot risico’s voor mens en milieu zal leiden, maar gezien de onvolledige moleculaire karakterisering kan zij vooralsnog niet positief adviseren over import en verwerking van MON89034.

De door de COGEM gehanteerde overwegingen en het hieruit voortvloeiende advies treft u hierbij aan als bijlage.

Hoogachtend,

Prof. dr. ir. Bastiaan C.J. Zoeteman
Voorzitter COGEM

c.c. Dr. D.C.M. Glandorf
Dr. I. van der Leij

# Import and processing of maize MON89034

## COGEM advice CGM/071022-02


**Summary**

*The present application by Monsanto S.A. of file EFSA/GMO/NL/2007/37, 'Lepidopteran pest resistant maize MON89034', concerns the import and processing for use in feed and food of a genetically modified maize line. Cultivation is not part of this application.*

*Maize line MON89034 has been genetically modified by insertion of the cry1A.105 and cry2Ab2 genes, which confer resistance to certain lepidopteran insects, such as the fall armyworm, the black cutworm and the corn earworm.*

*During the long domestication process, maize has lost its ability to survive in the wild. In the Netherlands, the appearance of maize volunteers is very 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. Therefore, COGEM is of the opinion that incidental spillage of maize line MON89034 will probably not pose a risk to human health and the environment. However, COGEM points out that the molecular analysis of maize line MON89034 is incomplete. In view of the incomplete molecular data, COGEM cannot advice positively on the application for import and processing of MON89034.*

*In addition, COGEM is of the opinion that the applicant should describe in more detail how the general surveillance plan will be organized. Furthermore, the applicant should ensure that information is obtained and that direct and indirect effects are reported annually.*


**Introduction**

The present application by Monsanto S.A. (file EFSA/GMO/NL/2007/37) concerns the import and processing of maize line MON89034 for use in feed and food. MON89034 contains the *cry1A.105* and *cry2Ab2* genes, which are constitutively expressed. The *cry1A.105* and *cry2Ab2* genes encode δ-endotoxins specific for insects of the order Lepidoptera. As a result, MON89034 is resistant to certain lepidopteran insects, such as the fall armyworm (*Spodoptera* sp.), the black cutworm (*Agrotis ipsilon*) and the corn earworm. *Cry* genes have been used in several genetically modified maize lines, which are cultivated throughout the world, but maize line MON89034 has not been cultivated in other countries and consequently has no history of safe use.

**Previous COGEM advices**

Although COGEM has advised several times on applications for maize genetically modified with other *cry* genes, COGEM has never advised on an organism genetically modified with the *cry1A.105* or the *cry2Ab2* genes.

**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 (1). Although maize is tolerant to a wide range of temperatures, it is typically grown in temperate regions due to the moisture level and the number of frost-free days required to reach maturity (1). Depending on cultivar and climate, the period from planting to harvesting ranges from 70 to 200 days (2). The minimum germination temperature is 10°C. Usually, the stem emerges from the soil four to six days after planting and flower initiation occurs 20 to 30 days after germination. The tassel of a four-month cultivar emerges 50 to 60 days after planting and the silk appears about a week later (2). Fertilization occurs through cross-pollination, and maize pollen is usually distributed by the wind (1). Hybridization with other species cannot occur as wild relatives of maize are not present in Europe (1).

During the long process of domestication, maize has lost the ability to survive in the wild and it needs human intervention to disseminate its seed (1). Maize kernels exhibit no dormancy and only survive under a narrow range of climatic conditions. Furthermore, maize is very sensitive to weed competition during the first four to six weeks after emergence (2) and it cannot persist as a weed (1). In the Netherlands, the appearance of volunteers is very rare and establishment of maize plants in the wild has never been observed.

**Construction and molecular characterization of MON89034**

*Transformation process*

*Agrobacterium tumefaciens*-mediated transformation was used to genetically modify maize line MON89034. *Agrobacterium tumefaciens* strain ABI contained the PV-ZMIR245 vector, which consisted of two T-DNA regions and the vector backbone. The T-DNA I region contains the *cry1A.105* and the *cry2Ab2* genes, which encode the Cry1A.105 and Cry2Ab2 proteins and provide protection to certain lepidopteran insects. The T-DNA II region contains the neomycin phosphotransferase II (*nptII*) gene, which confers resistance to certain aminoglycoside antibiotics, such as neomycin, kanamycin and paromycin. The T-DNA I and T-DNA II regions are both flanked by so-called right and left border sequences which allow the T-DNA regions to be inserted independently. After transformation paromycin resistant plants were selected. These plants contained the

T-DNA II region or the T-DNA I and II regions. During subsequent breeding the T-DNA I and T-DNA II regions which were integrated at different loci segregated. The plants that contained the T-DNA II region were eliminated and only the plants containing the T-DNA I region were selected.

The T-DNA I region consisted of:

- Right border region,
  right border region derived from the Ti-plasmid of *A. tumefaciens* used for transfer of the T-DNA;
- *e35S* promoter,
  promoter providing constitutive expression, which was derived from *Cauliflower mosaic virus* (CaMV) and contains the duplicated enhancer region;
- *Cab* leader,
  leader region from the chlorophyll a/b-binding protein from wheat;
- *Ract1* intron,
  intron from the rice actin gene;
- Cry1A.105 coding sequence,
  coding sequence for the *cry1A.105* gene which is a modified version of the *cry1A* gene from *Bacillus thuringiensis*. *Cry1A.105* encodes Cry1A.105, a modified Cry1A protein, which consists of domains I and II from Cry1Ab/Cry1Ac, domain III from Cry1F and substantially the entire C-terminal domain of Cry1Ac. The codon usage of *cry1A.105* has been optimized for expression in monocots.
- *Hsp17* terminator,
  terminator sequence from the wheat heat shock protein 17.3, which ends transcription and directs polyadenylation;
- *FMV* promoter,
  promoter providing constitutive expression from *Figwort Mosaic Virus* (FMV);
- *Hsp70* intron,
  intron from the heat shock protein 70 gene of maize;
- *SSU-CTP* targeting sequence,
  chloroplast targeting sequence of the small subunit of ribulose 1,5-bisphosphate carboxylase from maize;
- Cry2Ab2 coding sequence,

coding sequence for the *cry2Ab2* gene, which encodes the Cry2Ab2 protein. The Cry2Ab2 protein has been isolated from *B. thuringiensis* var. *kurstaki*. The codon usage of *cry2Ab2* has been optimized for expression in monocots;

- *nos* terminator,
  terminator sequence from the nopaline synthase gene of *A. tumefaciens,* which ends transcription and directs polyadenylation;
- Left border region,
  left border region derived from the Ti-plasmid of *A. tumefaciens* used for transfer of the T-DNA.

*Properties of the introduced genes*

Maize line MON89034 was genetically modified by the insertion of the *cry1A.105* and the *cry2Ab2* genes. The *cry1A.105* and *cry2Ab2* genes encode δ-endotoxins. These 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 midgut bacteria to enter the body cavity, which leads to septicemia and death (3). The δ-endotoxins which are encoded by the *cry1A.105* and *cry2Ab2* genes are specific for lepidopteran insects.

*Molecular analysis*

The applicant demonstrated by hybridization analysis that one copy of the T-DNA I region was integrated at a single integration site. Hybridization analyses performed on seven generations following transformation demonstrated that the insert was stable.

Results obtained by hybridization analyses performed with probes that span the vector backbone and the T-DNA II region showed that both the vector backbone and the T-DNA II region are absent in MON89034. Hybridization with a *nptII* probe confirmed that the *nptII* coding sequence is not present in MON89034.

PCR amplification of seven overlapping regions of the insert in MON89034 indicated that the arrangement of the elements in the T-DNA I region was maintained in MON89034. However, the results obtained by sequence analysis of the insert indicated that the e35S promoter was modified into a shorter version $e35S^{89}$. The $e35S^{89}$ promoter does not contain the duplicated enhancer element which is present in e35S. In addition, instead of the right border region a left border region was present. These results can be explained by a rearrangement between the T-DNA I and T-DNA II regions. The rearrangement did not affect any of the coding regions present in the insert and although the promoter of the *cry1A.105* gene was modified, the *cry1A.105* gene was still expressed. Since this rearrangement does not affect the introduced traits, COGEM is of

the opinion that the rearrangement does not affect the outcome of the risk analysis negatively.

Results obtained by PCR analysis of a conventional maize line using primers in the regions that flank the insert in MON89034 and results from the sequence comparison indicated that the regions flanking the insert in MON89034 are native to the maize genome. In addition, the sequence of the regions that flank the 5' and the 3' regions of the insert was compared to the sequence of a conventional maize line. Sequence analysis indicated that in the conventional maize line 57 basepairs were present at the location of the insertion in MON89034. These basepairs had been deleted in MON89034. In addition, ten basepairs had been added adjacent to the 5' region of the insert in MON89034. The junctions between the T-DNA and the maize genomic DNA were translated from stop codon to stop codon in all six reading frames. Bioinformatic analyses of the putative polypeptides in the junctions did not indicate any structural similarity to allergens or toxins. However, the large T-DNA border sequences at the 5' and the 3' end of the insert were not fully analyzed. COGEM is of the opinion that putative polypeptides present in the complete border sequences should be assessed for similarity to allergens or toxins.

Transformation of MON89034 was carried out with *A. tumefaciens* strain ABI in which the PV-ZMIR245 plasmid with the T-DNA I and T-DNA II regions had been introduced. In addition, strain ABI contained a helper plasmid without any T-DNA regions. However, it is unclear whether other plasmids, that may contain additional T-DNA regions, are present within ABI. COGEM is of the opinion that the applicant should provide information about the presence or absence of other plasmids and additional T-DNA regions in *A. tumefaciens* strain ABI.

**Environmental risk assessment**

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 rarely found and establishment of maize plants in the wild has never been observed. The applicant performed laboratory experiments and field studies to assess phenotype, agronomical characteristics and ecological interactions of MON89034. Studies that investigated ecological interactions reported qualitative instead of quantitative differences. These results do not indicate an increase in the potential of MON89034 to establish feral populations in the case of incidental spillage. However, COGEM wants to remark that qualitative data hampers a reliable statistical analysis. The lack of quantitative data on ecological interactions, thus hindering reliable statistical analyses, could pose a problem if this application would concern cultivation.

The results on phenotypic and agronomical characteristics, as well as on ecological interactions, indicated that there is no reason to assume that the expression of the *cry1A.105* and *cry2Ab2* genes in MON89034 increases the potential of maize to run wild.

**General surveillance plan**

A general surveillance plan is supplied by the applicant. General surveillance will be performed either by selected networks and/or specific company stewardship programs. However, the applicant does not indicate which networks or organizations will be involved in general surveillance and does not describe how the general surveillance will be organized. In addition, key stakeholders and networks are requested to participate in the general surveillance plan and are asked to inform the consent holder if any unanticipated adverse effects occur. However, it is unclear how these adverse effects are monitored if key stakeholders and networks do not assist. In addition, the applicant makes a distinction between reporting direct and indirect effects in the monitoring plan. According to the applicant direct effects will be reported annually, whereas indirect effects will only be reported at the stage of re-evaluation or at the end of a given permit. As stated in previous advices, COGEM is of the opinion that the applicant should report both direct and indirect effects annually.

In conclusion, in COGEM's opinion the applicant should describe in more detail how the general surveillance will be organized and should indicate which organizations are involved. In addition, the applicant should ascertain that information on adverse effects is obtained. Furthermore, direct and indirect effects should be reported annually.

**Advice**

COGEM has been asked to advice on import and processing for use in feed and food of maize line MON89034.

Maize has lost the ability to survive in the wild. In the Netherlands, volunteers are rarely found and establishment of maize plants in the wild has never been observed. In addition, maize needs human intervention to disseminate its seed. There is no reason to assume that the expression of the *cry1A.105* and *cry2Ab2* genes in MON89034 increases the potential of maize to establish feral populations. The genes that have been introduced 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 maize will not pose a risk to man and the environment.

A general surveillance plan has been provided by the applicant. However, it does not specify which organizations will be involved in general surveillance, nor does it describe how general surveillance will be organized. Moreover, it is unclear how the applicant ensures that information is obtained. In addition, the applicant proposes that indirect

effects will be reported at the stage of re-evaluation or at the end of a given permit. In COGEM's opinion the applicant should describe in more detail how the general surveillance will be organized and should indicate which particular organizations are involved. In addition, the applicant should ascertain that information on possible adverse effects is obtained. Furthermore, direct and indirect effects should be reported annually. Previously, COGEM has outlined the standards that have to be met by a post-market monitoring system and identified organizations which could be involved in post-market monitoring in the Netherlands (4).

In COGEM's view, it is sufficiently proven that only one copy of the T-DNA is present in MON89034. The applicant showed that the complete backbone of the plasmid and the T-DNA II region were absent. In addition, the applicant showed that the T-DNA was inserted in maize genomic DNA. Putative polypeptides in the junctions between the insert and the maize genomic DNA were studied and no homology to allergens or toxins was found. However, the analyses did not take into account the entire T-DNA border sequences. In addition, the applicant should provide information about the presence or absence of other plasmids and additional T-DNA regions in *A. tumefaciens* strain ABI, which has been used to genetically modify MON89034.

In view of the above, COGEM is of the opinion that import of MON89034 most likely poses a negligible risk to man and the environment. However, as the molecular characterization contains flaws, adverse effects could theoretically occur. Therefore, COGEM cannot advice positively on import and processing of maize line MON89034.

**References**


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