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**KENMERK** CGM/150528-01  
**ONDERWERP** Advies Import en verwerking van genetisch gemodificeerde 4114 maïs

Geachte mevrouw Mansveld,

Naar aanleiding van een adviesvraag betreffende import en verwerking van genetisch gemodificeerde maïs 4114 van Pioneer Hi-Bred International. Inc. (EFSA/GMO/NL/2014/123), deelt de COGEM u het volgende mee.

**Samenvatting:**

De COGEM is gevraagd om te adviseren over de mogelijke milieurisico's van import en verwerking van de genetisch gemodificeerde maïslijn 4114. Deze maïslijn brengt de genen *cry1F*, *cry34Ab1*, *cry35Ab1* en *pat* tot expressie. Hierdoor is de maïslijn resistent voor bepaalde insecten die behoren tot de ordes van de Lepidoptera en Coleoptera en tolerant voor glufosinaat-ammonium bevattende herbiciden.

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 ingebrachte genen ervoor zorgt dat de gg-maïs zou kunnen verwilderen. Bovendien zijn er in Europa geen wilde verwanten van maïs aanwezig waardoor uitkruising niet mogelijk is.

De COGEM acht de kans verwaarloosbaar klein dat incidenteel morsen tot verspreiding van deze maïslijn leidt. Verder is de COGEM van mening dat de moleculaire karakterisering adequaat is uitgevoerd.

Concluderend acht de COGEM de milieurisico's van import en verwerking van gg-maïs 4114 verwaarloosbaar klein. Omdat andere instanties een voedselveiligheidsbeoordeling uitvoeren, heeft de COGEM bij deze vergunningaanvraag de risico's van incidentele consumptie niet beoordeeld.



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

Hoogachtend,

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Voorzitter COGEM

c.c.           Drs. H.P. de Wijs, Hoofd Bureau ggo  
                  Mr. J.K.B.H. Kwisthout, Ministerie van IenM

# Import and processing of genetically modified maize 4114

## COGEM advice CGM/150528-01

### Summary

*The present application (EFSA/GMO/NL/2014/123) concerns import and processing for use in feed and food of genetically modified maize 4114. Cultivation is not part of this application.*

*Maize line 4114 was generated by Agrobacterium mediated transformation of the parental Pioneer proprietary maize line PHWWE and expresses the *pat*, *cry1F*, *cry34Ab1* and *cry35Ab1* genes. As a result, maize 4114 is tolerant to glufosinate-ammonium containing herbicides, and resistant to certain lepidopteran and coleopteran insects.*

*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 allow 4114 maize to establish feral populations. The introduced genes cannot spread to closely related species since wild relatives of maize are not present in Europe.*

*The applicant showed by Southern blot analyses that maize line 4114 contains one copy of the insert at a single integration locus and that the backbone of the plasmid used for transformation is absent.*

*Bioinformatic analysis of the junctions of the inserted sequences and the maize genomic DNA, identified eleven open reading frames (ORFs). These ORFs were analysed for similarity to known proteins or allergens. No similarities to allergens and toxins were found. The molecular characterization of maize 4114 meets the criteria of COGEM. COGEM considers the appended General Surveillance plan adequate for import of maize 4114.*

*In conclusion, COGEM is of the opinion that incidental spillage of maize 4114 poses a negligible risk to the environment. COGEM abstains from giving advice on the potential risks of incidental consumption since a food/feed assessment is already carried out by other organizations. COGEM considers the environmental risks associated with import and processing of maize 4114 to be negligible.*

### Introduction

The scope of the present notification by Pioneer Hi-Bred International Inc. concerns import and processing of 4114 maize for use in feed and food. The genetically modified maize is produced by *Agrobacterium tumefaciens* mediated transformation of Pioneer proprietary maize line PHWWE and expresses the *pat* gene conferring tolerance to glufosinate-ammonium containing herbicides and three *cry* genes. The *cry1F* gene provides resistance to some lepidopteran insects such as the European corn borer (*Ostrinia nubilalis*). The two *Cry* proteins *Cry34Ab1* and *Cry35Ab1* act together as binary toxins to confer resistance to certain coleopteran insects such as the corn rootworm (*Diabrotica virgifera*).

### **Previous COGEM advice**

COGEM advised positively on import and processing of hybrid maize 59122 x 1507 expressing two *pat* genes and three *cry* genes, *cry1F*, *cry34Ab1* and *cry35Ab1*. These genes are also present in maize 4114.<sup>1</sup> COGEM has also given positive advice on the cultivation of maize line 1507 that expresses the *pat* and *cry1F* genes.<sup>2</sup>

### **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. Maize is predominantly wind pollinated.<sup>3,4</sup> Although pollination by bees and other insects cannot be completely excluded, insect pollination is limited since the female flowers do not produce nectar and are therefore not attractive to insect pollinators.<sup>5</sup> In Europe, no wild relatives of maize are present and therefore hybridisation with other species cannot occur.

In the Netherlands, the appearance of volunteers is very rare to absent.<sup>6</sup> Domesticated maize requires warm conditions in order to grow and does not tolerate prolonged cold and frost.<sup>5,7</sup> The seeds (kernels) remain on the cob after ripening and do not shatter naturally.<sup>5,8</sup> In cultivation areas with warmer climatic conditions, the appearance of volunteers can occur the year following maize cultivation due to spilled cobs or kernels. However, these volunteers are usually killed by common mechanical pre-planting soil preparation practices.<sup>5</sup> Maize is very sensitive to weed competition.<sup>9</sup>

During the long process of domestication, maize has lost the ability to survive in the wild.<sup>4</sup> 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 characterization**

Maize 4114 was produced by *Agrobacterium tumefaciens* (disarmed strain LBA4404) mediated transformation of the parental line using binary plasmid PHP27118. This plasmid contains *cry1F*, *cry34Ab1* and *cry35Ab1* expression cassettes, which were inserted from the plasmids previously used to generate 1507 and 59122 maize.

An overview of the T-DNA introduced in 4114 maize (11,978 basepairs (bp)) is given below:

- T-DNA Right border region of *A. tumefaciens*
- Ti plasmid region. Non-functional sequence from Ti plasmid of *A. tumefaciens*.
- Polylinker region. Sequence used in DNA cloning.
- *ubiZM1* promoter. Promoter region from *Z. mays* polyubiquitin gene.
- *ubiZM1* 5'UTR. 5' untranslated region from *Z. mays* polyubiquitin gene.
- *ubiZM1* intron. Intron region from *Z. mays* polyubiquitin gene.
- Polylinker Region. Sequence used in DNA cloning.
- *cry1F* gene. Truncated version of the *cry1F* gene from *Bacillus thuringiensis* var. *aizawai*
- Polylinker Region. Sequence used in DNA cloning.
- ORF 25 terminator. Terminator sequence from *A. tumefaciens* pTi15955 ORF 25.
- Polylinker Region. Sequence used in DNA cloning.

- *ubiZM1* promoter. Promoter region from *Z. mays* polyubiquitin gene.
- *ubiZM1* 5'UTR. 5' untranslated region from *Z. mays* polyubiquitin gene.
- *ubiZM1* intron. Intron region from *Z. mays* polyubiquitin gene.
- Polylinker Region. Sequence used in DNA cloning.
- *cry34Ab1* gene. Synthetic version of the *cry34Ab1* gene encoding the 14 kDa delta-endotoxin parasporal crystal protein from the non-motile strain PS149B1 of *Bacillus thuringiensis*.
- Polylinker Region. Sequence used in DNA cloning.
- *pinII* terminator. Terminator region from *Solanum tuberosum* proteinase inhibitor II gene.
- Polylinker Region. Sequence used in DNA cloning.
- TA peroxidase promoter. Promoter from *Triticum aestivum* peroxidase including leader sequence
- Polylinker Region. Sequence used in DNA cloning.
- *cry35Ab1* gene. Synthetic version of the *cry35Ab1* gene encoding a 44 kDa delta-endotoxin parasporal crystal protein from the non-motile strain PS149B1 of *B. thuringiensis*.
- Polylinker Region. Sequence used in DNA cloning.
- *pinII* terminator. Terminator region from *Solanum tuberosum* proteinase inhibitor II gene.
- Polylinker Region. Sequence used in DNA cloning.
- CaMV 35S promoter. 35S promoter from *Cauliflower Mosaic Virus*.
- Polylinker Region. Sequence used in DNA cloning.
- *pat* gene. Synthetic, plant-optimized phosphinothricin acetyltransferase coding sequence from *Streptomyces viridochromogenes*.
- Polylinker Region. Sequence used in DNA cloning.
- CaMV35S terminator. 35S terminator from *Cauliflower Mosaic Virus*.
- Polylinker Region. Sequence used in DNA cloning.
- Ti plasmid region. Non-functional sequence from Ti plasmid of *A. tumefaciens*.
- T-DNA Left border region of *A. tumefaciens*.

### ***Herbicide tolerance trait***

Maize 4114 expresses the *pat* gene from *Streptomyces viridochromogenes* that encodes phosphinothricin acetyltransferase (PAT). Expression of PAT confers tolerance to glufosinate-ammonium herbicides.<sup>10</sup> The active ingredient in glufosinate-ammonium herbicide is L-phosphinothricin (L-PPT), which binds to glutamine synthetase in plants. The detoxification of excess ammonia is thereby prevented, leading to plant death. Maize 4114 produces PAT which catalyses the conversion of L-PPT to an inactive form, which does not bind glutamine synthetase, therefore maize 4114 is still able to detoxify ammonia even if glufosinate-ammonium is used.<sup>11</sup>

### ***Insect resistance traits***

Maize 4114 expresses the *cryIF*, *cry34Ab1* and *cry35Ab1* genes. These genes encode  $\delta$ -endotoxins. *CryIF* is derived from *Bacillus thuringiensis* var. *aizawai* and is lethal to certain insects of the lepidopteran order, including larvae of the European corn borer (*Ostrinia nubilalis*) and the pink corn borer (*Sesamia cretica*). The *cry34Ab1* and *cry35Ab1* genes are derived from *B. thuringiensis*

strain PS149B1. These two genes act together as a binary pair that acts synergistically to control certain coleopteran pests (rootworms).

### ***Molecular analysis***

The applicant demonstrated by Southern blot analyses that the 4114 maize contains one copy of the insert at a single integration locus and that the backbone sequences of plasmid PHP27118 are absent.

The applicant determined the sequence of the insert and adjacent flanking genomic DNA sequences (>2kb) in maize 4114. The T-DNA is inserted in a region coding for a hypothetical glutaredoxin-like protein. The applicant showed by Northern blot analysis that this hypothetical gene is not expressed in the parental line. Thus, the inserted T-DNA does not disrupt the function of an active endogenous gene.

A comparison with the parental maize line revealed that 24 bp were introduced at the 5' end of the inserted T-DNA. According to the applicant these sequences were identical to T-DNA sequences. The comparison also revealed that the Right border of the T-DNA was deleted completely together with 4 bp of non-functional T-DNA sequence. The Left border of the T-DNA was almost completely deleted, 1 bp remained. The remaining sequence was identical to the T-DNA present in plasmid PHP27118. Bioinformatic analysis showed that the 5' and 3' flanking sequences of the integrated DNA are maize genomic DNA. The remaining sequence was identical to the T-DNA present in plasmid PHP27118. Bioinformatic analysis showed that the 5' and 3' flanking sequences of the integrated DNA are maize genomic DNA.

The applicant screened the junctions between the T-DNA insert and the flanking plant genomic DNA for potential newly created open reading frames (ORFs). Eleven ORFs potentially encoding peptides of eight amino acids or more were identified. The amino acid sequences were compared to a dataset of known and putative allergen sequences (FARPP dataset, January 2014) and to a publicly available protein dataset (NCBI Entrez Protein dataset, February 2014) using the BLASTP algorithm to identify possible similarities to known allergens or toxins. The applicant states that no similarities to allergens or toxins were identified.

### **Food/ feed assessment**

COGEM abstains from giving advice on the potential risks of incidental consumption since a food/feed assessment is already carried out by other organisations.<sup>12</sup> This application is submitted under Regulation (EC) 1829/2003, therefore a food/feed assessment is carried out by EFSA and national organisations involved in the assessment of food safety. 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 when this advice was completed.

### **General surveillance**

General surveillance (GS) has been introduced to be able to observe unexpected adverse effects of genetically modified (GM) crops on the environment. A GS plan is required for every application for market authorisation.

The current GS plan states that unanticipated adverse effects will be monitored by existing monitoring systems, which include the authorisation holder and operators involved in the handling and use of viable maize. The third parties (operators) involved in GS will report adverse effects to the authorisation holder.

In 2010, COGEM published a report on the principles that, according to COGEM, should be followed for general surveillance.<sup>13</sup> COGEM considers the submitted GS plan adequate for import of 4114 maize however, the plan could be improved on the following points.

In the present GS plan, the authorisation holder states that the operators have agreed to provide information relevant to the monitoring of 4114 maize to the authorisation holder. The GS plan could be improved by a guarantee that operators will monitor for unanticipated effects. In particular a statement is lacking that the authorisation holder will give evidence that the operators collect this information.

The GS plan further 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.

In the EFSA guidance document, EFSA states that the applicant should make raw data and analysis of monitoring data available to the Competent Authorities and the European Commission.<sup>14</sup> COGEM agrees with this request and points out that the applicant should include a statement on this point in the GS plan.<sup>15</sup>

### **Considerations and advice**

COGEM has been asked to advise on import and processing for use in food and feed of herbicide tolerant and insect resistant maize line 4114. This genetically modified maize line expresses the *pat*, *cry1F*, *cry34Ab1* and *cry35Ab1* genes providing tolerance to glufosinate-ammonium containing herbicides and resistance against certain lepidopteran and coleopteran insect pests.

COGEM is of the opinion that the risk of spread of 4114 maize within the Netherlands due to incidental spillage of the maize line is negligible. Wild relatives of maize are not present in Europe and therefore introduction of the inserted genes into close related species cannot occur. Although the GS plan could be improved, COGEM considers the submitted GS plan adequate for import of 4114 maize.

Based on the aspects discussed, COGEM is of the opinion that import and processing of 4114 maize poses a negligible risk to the environment in the Netherlands. A food/feed safety assessment is carried out by other organisations. Therefore, COGEM abstains from advice on the potential risks of incidental consumption.

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