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DATUM	9 juli 2021
KENMERK	CGM/210709-01
ONDERWERP	Advies import en verwerking van de gg-maïslijn DP4114xMON89034xMON87411xDAS-40278-9

Geachte mevrouw Van Nieuwenhuizen,

Naar aanleiding van een vergunningaanvraag voor import en verwerking van genetisch gemodificeerde maïs DP4114xMON89034xMON87411xDAS-40278-9 (EFSA/GMO/NL/2020/171), ingediend door Pioneer Hi-bred International, Inc., deelt de COGEM u het volgende mee.

Samenvatting:
De COGEM is gevraagd te adviseren over de mogelijke milieurisico's van import en verwerking van genetisch gemodificeerde (gg-) maïs DP4114xMON89034xMON87411xDAS-40278-9 en alle subcombinaties daarvan. Deze gg-mais bevat een *snf7* genconstruct en de genen *cry1F*, *cry34Ab1*, *cry35Ab1*, *cry1A.105*, *cry2Ab2*, *cry3Bb1*, *cp4 epsps*, *pat* en *aad-1*tot expressie, waardoor de maïsplanten resistent tegen bepaalde plaaginsecten en tolerant voor bepaalde herbiciden zijn. De moleculaire karakterisering voldoet aan de eisen van de COGEM.
Verwildering van maïsplanten is in Nederland nooit waargenomen. Bovendien komt de wilde verwant van maïs (teosinte) niet in Nederland voor, waardoor de ingebrachte sequenties zich niet naar andere soorten kunnen verspreiden. Er zijn geen redenen om aan te nemen dat expressie of transcriptie van de ingebrachte genen ervoor zorgt dat deze gg-maïslijn zich in Nederland zou kunnen vestigen of zou kunnen verwilderen. Ook zijn er geen redenen om aan te nemen dat import en verwerking van deze gg-maïslijn tot nadelige effecten voor het milieu in Nederland zal leiden. De COGEM acht de milieurisico's bij import en verwerking van DP4114xMON89034x MON87411xDAS-40278-9, en sub-combinaties hiervan, verwaarloosbaar klein.
Omdat een voedselveiligheidsbeoordeling door andere instanties wordt uitgevoerd, 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,

Prof. dr. ing. Sybe Schaap
Voorzitter COGEM

c.c.
- Drs. Y. de Keulenaar, Hoofd Bureau ggo
- Ministerie van IenW, Directie Omgevingsveiligheid en Milieurisico's DG Milieu en Internationaal
- Ing. M.A.C. Möllers, Food-Feed loket

Environmental risk assessment of import and processing of genetically modified maize DP4114xMON89034xMON87411xDAS-40278-9 and its sub-combinations

COGEM advice CGM/210709-01

- The present application (EFSA/GMO/NL/2020/171) concerns the authorisation for import and processing for use in feed and food of genetically modified (GM) maize DP4114xMON89034xMON87411xDAS-40278-9 and its sub-combinations;
- The stacked event DP4114xMON89034xMON87411xDAS-40278-9 was produced by conventional crossbreeding of the four GM parental maize lines;

- The GM maize expresses Cry1F, Cry34Ab1, Cry35Ab1, Cry1A.105, Cry2Ab2, Cry3Bb1, CP4 EPSPS, PAT and AAD-1 proteins and contains DvSnf7 double-stranded ribonucleic acid (dsRNA), conferring resistance to certain lepidopteran and coleopteran pests, and tolerance to glyphosate, glufosinate-ammonium, 2,4-dichlorophenoxyacetic acid (2,4-D) and aryloxyphenoxypropionate (AOPP) containing herbicides.

- In the Netherlands, feral maize populations have never been observed and the appearance of volunteers is rare;
- In the Netherlands, the wild relative of maize, teosinte is not present in the natural environment, hybridisation of GM maize with other species is therefore not possible;

- The molecular characterisation of DP4114xMON89034xMON87411xDAS-40278-9 has been updated and meets the criteria of COGEM;
- The updated bioinformatic analyses do not provide indications for potential environmental risks;

- There are no indications that the introduced traits allow the stacked event maize DP4114xMON89034xMON87411xDAS-40278-9 to survive in the Netherlands;
- There are no indications that DP4114xMON89034xMON87411xDAS-40278-9 could establish feral populations in the Netherlands;

- COGEM is of the opinion that import and processing of maize DP4114xMON89034xMON87411xDAS-40278-9 and GM maize consisting of sub-combinations of its parental GM maize lines pose a negligible risk to the environment in the Netherlands;
- COGEM abstains from giving advice on the potential risks of incidental consumption since a food/feed assessment is carried out by other organisations.

1. Introduction

The present application (EFSA/GMO/NL/2020/171), filed by Pioneer Hi-bred International, Inc., concerns the import and processing of genetically modified (GM) maize DP4114x MON89034xMON87411xDAS-40278-9 and GM maize consisting of its sub-combinations for use in feed and food. The GM maize was produced by conventional crossbreeding of the four GM parental maize lines. It expresses the Cry1F, Cry34Ab1, Cry35Ab1, Cry1A.105, Cry2Ab2, Cry3Bb1, CP4 EPSPS, PAT and AAD-1 proteins and the DvSnf7 double-stranded ribonucleic acid (dsRNA), conferring resistance to certain lepidopteran and coleopteran pests, and tolerance to glyphosate, glufosinate-ammonium, 2,4-dichlorophenoxyacetic acid (2,4-D) and aryloxyphenoxypropionate (AOPP) containing herbicides.

The parental lines DP4114[1], MON89034[2], MON87411[3] and DAS-40278-9[4] have all been authorised for import and processing for use in food and feed in the European Union.

2. Previous COGEM advice

Previously COGEM advised positively on the import and processing of the parental lines DP4114[5], MON89034[6], MON87411[7] and DAS-40278-9[8]. COGEM also advised positively on the import and processing of several stacked events containing parental lines of the stacked event under assessment, including MON89034x1507xMIR162xNK603xDAS-40278-9[9] and MON87427xMON89034x1507 xMON87411x59122xDAS-40278-9.[10]

3. Environmental risk assessment

3.1 Characteristics of maize

Maize (*Zea mays*) is a member of the grass family *Poaceae*. It is a highly domesticated crop originating from Central America, but nowadays cultivated globally. Maize is wind pollinated[11,12] and has both male and female flowers that are spatially separated. The female flowers are not attractive to insect pollinators, because they do not produce nectar. Insect pollination of maize is highly limited but cannot be excluded.[13]

Hybridisation of GM maize with other species than teosinte, the wild relative of maize, cannot occur.

Maize requires warm conditions in order to grow.[13,14] In cultivation areas with warm 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.[13] Maize does not tolerate prolonged cold and frost.[15]

Maize is very sensitive to weed competition.[16] During the long process of domestication, maize has lost the ability to persist in the wild.[12] A soil seed bank, small seeds, and an extended period of flowering and seed production are characteristics often observed in persistent weeds.[17] Maize lacks all these characteristics. After ripening, the seeds (the kernels) adhere to the cob and do not scatter naturally.[13,18] Consequently, seed dispersal is severely hampered.

3.2 Receiving environment

In the Netherlands, the appearance of maize volunteers is rare, although maize plants occasionally have been observed outside agricultural fields.[19,20] Any volunteers emerging will be killed by frost at the onset of winter.[15] COGEM is not aware of any reports of feral maize populations in the Netherlands. Additionally, hybridisation of GM maize with teosinte is not possible, as teosinte is absent in maize fields and in nature in the Netherlands.[15]

Conclusion: In the Netherlands, feral maize populations do not occur and hybridisation of maize with other species is impossible.

3.2 Description of the introduced genes, traits and regulatory elements

Maize DP4114xMON89034xMON87411xDAS-40278-9 was created by conventional cross-breeding of the parental lines. For a description of the parental lines, see previous COGEM advices.[5,6,7,8] A description of the inserted genetic elements is listed in the table below. The list is limited to information on the introduced genes, corresponding traits, and regulatory elements (promoters and terminators).

Introduced genes	**Encoded proteins**	**Traits**	**Regulatory elements**
cry1F (DP4114)	Truncated Cry1F protein originating from *Bacillus thuringiensis* var. *aizawai*[21]	Resistance to certain lepidopteran insects	Polyubiquitin (*ubi*ZM1) promoter from *Zea mays* and ORF25 terminator from *Agrobacterium tumefaciens* strain pTi15955
cry34Ab1 (DP4114)	Cry34Ab1 protein originating from *B. thuringiensis* strain PS149B1[22,23,24,25,26]	Resistance to certain lepidopteran insects	*Ubi*Zm1 promoter from *Z. mays* and proteinase inhibitor II (*pin*II) terminator from *Solanum tuberosum*
cry35Ab1 (DP4114)	Cry35Ab1 protein originating from *B. thuringiensis* strain PS149B1[22,23,24,25,26]	Resistance to certain lepidopteran insects	TA peroxidase promoter from *Triticum aestivum* and *pin*II terminator from *S. tuberosum*
cry1A.105 (MON89034)	The Cry1A.105 protein is a chimeric protein with domains from different Cry1 proteins from *B. thuringiensis* subsp. *kumamotoensis*[27]	Resistance to certain lepidopteran insects	Enhanced *35S* (*e35S*) promoter from *Cauliflower mosaic virus* (CaMV) and T-Hsp17 terminator from *Triticum aestivum*
cry2Ab2 (MON89034)	Variant of the Cry2Ab2 protein from *B. thuringiensis* subsp. *kurstaki*[27]	Resistance to certain lepidopteran insects	Promoter from *Figwort mosaic virus* (FMV) and nopaline synthase (*nos*)

			terminator from *A. tumefaciens*
cry3Bb1 (MON87411)	Variant of the Cry3Bb1 protein originating from *B. thuringiensis* subsp. *kumamotoensis*[28]	Resistance to certain coleopteran insects	PIIG promoter from *Z. mays* and Hsp17 3'UTR from *T. aestivum*
cp4 epsps (MON87411)	The 5-enolpyruvulshikimate-3-phosphate synthase (EPSPS) enzyme originating from *A. tumefaciens* strain CP4[29]	Tolerance to glyphosate containing herbicides	Enhanced *35S* (*e35S*) promoter from *Cauliflower mosaic virus* (CaMV) and nopaline synthase (*nos*) terminator from *A. tumefaciens*
pat (DP4114)	Plant optimised version of phosphinothricin acetyl transferase (PAT) from *Streptomyces viridochromogenes* strain Tü 494[30,31,32]	Tolerance to glufosinate-ammonium containing herbicides	*35S* promoter and *35S* terminator from *Cauliflower mosaic virus* (CaMV)
aad-1 (DAS-40278-9)	Aryloxyalkanoate dioxygenase (AAD-1) enzyme originating from *Sphingobium herbicidovorans*[33]	Tolerance to several synthetic auxin acting herbicides like 2,4 dichlorophenoxyacetic acid' (2,4-D) and to AOPP containing herbicides	Polyubiquitin promoter (Zm*Ubi*Int) and Zmper5 3'UTR terminator from *Z. mays*
DvSnf7b (MON87411)	Expresses a dsRNA transcript that contains a fragment of the *Snf7* gene from *Diabrotica virgifera virgifera*[34]	Resistance to certain coleopteran insects	Enhanced *35S* (*e35S*) promoter from *Cauliflower mosaic virus* (CaMV) and E9 gene 3'UTR from *Pisum sativum*
For a detailed description of the introduced genes and traits, see references			

3.3 Molecular characterisation

Previously, COGEM evaluated the molecular characterisation of each parental line and considered these to be adequate.[5,6,7,8]

The integrity and copy number of the inserts in DP4114xMON89034xMON87411xDAS-40278-9 were confirmed by Southern blot analysis and were determined to be equivalent to the inserts in the corresponding parental lines. The applicant resequenced the inserts and their flanking regions and compared the obtained sequences with the sequences of the corresponding parental lines. According

to the applicant, the insert and flanking regions in the stacked maize event are identical to the sequences determined previously for the respective parental lines.

The applicant updated the bioinformatics analyses of the inherited inserted elements and the sequences spanning the insertion sites and 5' and 3' flanking regions using recent databases. According to the applicant, the putative products of the open reading frames spanning the 5' and 3' junctions of the inserts, and those within the inserts themselves, did not generate any protein sequence similarity with known allergens or toxins.

The molecular characterisation was conducted according to the criteria previously laid down by COGEM.[35] The results from the updated molecular characterisation do not provide indications that the stacked event maize DP4114xMON89034xMON87411xDAS-40278-9 could pose a risk to the environment.

Conclusion: The molecular characterisation of the stacked event maize DP4114xMON89034xMON87411xDAS-40278-9 is adequate and no indications for potential environmental risks were identified

3.4 Phenotypic and agronomic characteristics

Previously, COGEM evaluated the phenotypic and agronomic characteristics of each parental line of DP4114xMON89034xMON87411xDAS-40278-9, and found no deviations between the parental line and the conventional control influencing the outcome of the environmental risk assessment.

The applicant analysed the phenotypic and agronomic characteristics of DP4114xMON89034x MON87411xDAS-40278-9 and noted that these, except for the introduced traits, are not different from the non-GM control (conventional counterpart), and are equivalent to the reference varieties, taking into account natural variation. The results of the phenotypic and agronomic evaluation do not give reason to assume that the GM maize could pose an environmental risk.

In conclusion, COGEM is of the opinion that there are no reasons to assume that the introduced traits in DP4114xMON89034xMON87411xDAS-40278-9 allow it or its sub-combinations to survive or establish in the Dutch environment.

Conclusion: There are no indications that the introduced traits allow DP4114xMON89034x MON87411xDAS-40278-9 or its sub-combinations to survive in the Netherlands or that this GM maize line has an increased potential to establish feral populations in the Netherlands.

4. Food/feed assessment

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 Wageningen Food Safety Research (WFSR). The outcome of the assessment by other organisations (EFSA, WFSR) was not known when this advice was completed.

5. Post-market environmental monitoring (PMEM)

The applicant supplied a general surveillance (GS) plan as part of the post-market environmental monitoring (PMEM). COGEM has published several recommendations for further improvement of the general surveillance (GS) plan,[36,37] but considers the current GS plan adequate for the import and processing of maize DP4114xMON89034xMON87411xDAS-40278-9.

6. Overall conclusion

Conclusion: COGEM is of the opinion that import and processing of DP4114xMON89034xMON87411xDAS-40278-9 maize and GM maize consisting of sub-combinations of the stacked maize line poses a negligible risk to the environment in the Netherlands.
COGEM abstains from giving advice on the potential risks of incidental consumption since other organisations carry out a food/feed assessment.

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