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KENMERK CGM/180806-03
ONDERWERP Advies import en verwerking van gg-mais
MON87427xMON89034x1507xMON87411x59122xDAS-40278-9

Geachte mevrouw Van Veldhoven,

Naar aanleiding van een vergunningaanvraag voor import en verwerking van genetisch gemodificeerde maïs MON87427xMON89034x1507xMON87411x59122xDAS-40278-9 (EFSA/GMO/NL/2017/145), ingediend door Dow AgroSciences LLC, deelt de COGEM u het volgende mee.

Samenvatting:

De COGEM is gevraagd te adviseren over de mogelijke milieurisico's van import en verwerking van de genetisch gemodificeerde (gg-) maïs MON87427xMON89034x1507xMON87411x59122xDAS-40278-9 en alle subcombinaties daarvan. In deze gg-maïs komen de genen *cp4 epsps*, *pat* en *aad-1* tot expressie, waardoor de plant tolerant is voor bepaalde herbiciden. Ook komen de genen *cry1A.105*, *cry2Ab2*, *cry34Ab1*, *cry35Ab1*, *cry1F*, *cry3Bb1* en dsRNA *DvSnf7* tot expressie, waardoor de plant resistent is tegen bepaalde plaaginsecten die behoren tot de vlinder- en keverachtigen.

Verwildering van maïsplanten is in Nederland nooit waargenomen. Maïsplanten uit gemorst zaad (opslagplanten) worden hier nauwelijks aangetroffen. Bovendien zijn er in Nederland geen wilde verwanten van maïs aanwezig, waardoor de ingebrachte sequenties niet naar andere soorten kunnen verspreiden.

De COGEM merkt op dat de moleculaire karakterisering van MON87427xMON89034x1507xMON87411x59122xDAS-40278-9 onvolledig is. De bioinformatische analyses zijn niet uitgevoerd met de meest recente sequentie informatie. De COGEM is van oordeel dat herziene gegevens door de aanvrager overlegd moeten worden, teneinde een volledige analyse van de risico's voor mens en milieu te kunnen uitvoeren.

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. H.P. de Wijs, Hoofd Bureau ggo
 Mr. J.K.B.H. Kwisthout, Ministerie van IenW
 Ing. M.A.C. Möllers, Food-Feed loket

**Import and processing of genetically modified maize
MON87427xMON89034x1507xMON87411x59122xDAS-40278-9
and sub-combinations**

COGEM advice CGM/180806-03

- The present application (EFSA/GMO/NL/2017/145) concerns the authorisation for import and processing for use in feed and food of genetically modified (GM) maize MON87427xMON89034x1507xMON87411x59122xDAS-40278-9 and GM maize consisting of sub-combinations of the parental GM maize lines;
- Maize MON87427xMON89034x1507xMON87411x59122xDAS-40278-9 was produced by conventional crossbreeding of the six GM parental maize lines;
- Previously, COGEM advised positively on the import and processing of all six parental lines;

- The GM maize expresses the genes *cp4 epsps*, *pat*, *aad-1*, *cry1A.105*, *cry2Ab2*, *cry34Ab1*, *cry35Ab1*, *cry1F*, *cry3Bb1* and dsRNA *DvSnf7*;
- It is resistant to certain lepidopteran and coleopteran insects, and tolerant to 2,4-D, AOPP, glyphosate and glufosinate-ammonium containing herbicides;

- In the Netherlands, feral maize populations have never been observed and the appearance of volunteers is rare;
- In the Netherlands, wild relatives of maize are absent and hybridisation of maize with other species is therefore not possible;

- COGEM considers the molecular characterisation of MON87427xMON89034x1507xMON87411x59122xDAS-40278-9 incomplete;
- New sequence information showed differences between stacked and parental lines, and updated bioinformatics analyses were not performed on the most recent sequence information;

- There are no indications that the introduced traits allow MON87427xMON89034x1507xMON87411x59122xDAS-40278-9 to survive in the Netherlands;
- There are no indications that MON87427xMON89034x1507xMON87411x59122xDAS-40278-9 could establish feral populations in the Netherlands;

- COGEM is of the opinion that the dossier of maize MON87427xMON89034x1507xMON87411x59122xDAS-40278-9 is incomplete, and deems it necessary that the bioinformatics analyses should be performed on updated sequence information before a risk assessment can be made;
- 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/2017/145) filed by Dow AgroSciences LLC concerns import and processing of genetically modified (GM) maize MON87427xMON89034x1507xMON87411x59122xDAS-40278-9 and GM maize consisting of sub-combinations of the parental lines. The GM maize was produced by conventional crossbreeding of six GM parental maize lines. It expresses the *cp4 epsps*, *pat* and *aad-1* genes, which confer tolerance to 2,4-D, AOPP, glyphosate and glufosinate-ammonium containing herbicides. It also expresses the *cry1a.105*, *cry2Ab2*, *cry34Ab1*, *cry35Ab1*, *cry1F*, *cry3Bb1* and dsRNA *DvSnf7* genes resulting in resistance to certain lepidopteran and coleopteran insects.

Five parental lines, MON87427¹, MON89034², 1507³, 59122⁴ and DAS-40278-9⁵, have been authorised for import and processing for use in food and feed in the European Union. The European Food Safety Authority (EFSA) has issued a positive opinion on import and processing of maize MON87411 in May 2018⁶, but the European Commission has not yet authorized import of MON87411.

2. Previous COGEM advice

COGEM has previously advised positively on import and processing of all six parental lines: MON87427⁷, MON89034⁸, 1507^{9,10}, MON87411¹¹, 59122^{12,13}, and DAS-40278-9¹⁴. COGEM also advised positively on the import and processing of several stacked events, including 1507x59122¹⁵, MON87427xMON89034x1507xMON88017x59122¹⁶, and MON89034x1507xMON88017x59122xDAS-40278-9¹⁷. The environmental risks associated with import and processing were considered negligible.

3. Environmental risk assessment

Potential environmental risks of MON87427xMON89034x1507xMON87411x59122xDAS-40278-9 maize and of GM maize consisting of sub-combinations of its parental GM maize lines are assessed as part of the environmental risk assessment carried out by COGEM.

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,^{18,19} and has both male and female flowers that are spatially separated. Female flowers are not attractive to insect pollinators, because they do not produce nectar. Insect pollination of maize is probably highly limited but cannot be excluded.²⁰

Recently, the wild relative of maize teosinte has been reported as a weed in maize fields in Spain^{21,22,23} and France.^{24,25} In the Netherlands, no wild relatives of maize are present and hybridisation with other species cannot occur.

Maize requires warm conditions in order to grow and does not tolerate prolonged cold and frost.^{20,26} 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.²⁰

Maize is very sensitive to weed competition.²⁷ During the long process of domestication, maize has lost the ability to persist in the wild.¹⁹ A soil seed bank, small seeds, and an extended period of flowering and seed production are characteristics often observed in persistent weeds.²⁸ Maize lacks all these characteristics. After ripening, the seeds (the kernels) adhere to the cob and do not shatter naturally.^{20,29} Consequently, seed dispersal is severely hampered.

During field observations in Austria some volunteers and maize plants were observed in non-agricultural habitats.³⁰ In the Netherlands, the appearance of volunteers is very rare, although maize plants occasionally have been observed outside agricultural fields.³¹ COGEM is not aware of any reports of feral maize populations in the Netherlands or elsewhere in Europe.

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

3.2 Description of the introduced genes, traits and regulatory elements

Maize MON87427xMON89034x1507xMON87411x59122xDAS-40278-9 was created by conventional crossbreeding of the parental lines. For a detailed description of the parental lines, see previous COGEM advises.^{7,8,9,10,11,12,13,14,32} A description of the inserted genetic elements are 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
<i>cp4 epsps</i> (two copies)	The 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme originating from <i>Agrobacterium tumefaciens</i> strain CP4 ^{7,11,33}	Tolerance to glyphosate containing herbicides	<i>Cauliflower mosaic virus</i> (CaMV) e35S promoter and T-nos terminator from <i>A. tumefaciens</i> derived from MON87427. <i>TubA</i> promoter and 3'UTR from <i>Oryza sativa</i> derived from MON87411
<i>Pat</i> (two copies)	Variant of phosphinothricin N-acetyltransferase (PAT) originating from <i>Streptomyces viridochromogenes</i> strain Tü 494 ^{9,10,12,13,34,35}	Tolerance to glufosinate-ammonium containing herbicides	CaMV 35S promoter and CaMV 35S terminator derived from both 1507 and 59122
<i>aad-1</i>	Aryloxyalkanoate dioxygenase (AAD-1) enzyme originating from	This enzyme can cleave several synthetic auxins, and certain 'aryloxyphenoxy-	ZmUbi1 promoter and Zmper5 3'UTR terminator from <i>Zea</i>

Introduced genes	Encoded proteins	Traits	Regulatory elements
	<i>Sphingobium herbicidovorans</i> ^{14,36}	propionate' (AOPP) herbicides, resulting in a tolerance to several synthetic auxin acting herbicides like 2,4 dichlorophenoxyacetic acid' (2,4-D) and to AOPP containing herbicides	<i>mays</i>
<i>cry1A.105</i>	The Cry1A.105 protein is a chimeric protein with domains from different Cry1 proteins from <i>Bacillus thuringiensis</i> ^{8,32,37}	Resistance to certain lepidopteran insects	CaMVe35S promoter and T-Hsp17 terminator from <i>Triticum aestivum</i>
<i>cry2Ab2</i>	Variation of the Cry2Ab2 protein from <i>B. thuringiensis</i> subsp. <i>kurstaki</i> ^{8,32,37}	Resistance to certain lepidopteran insects	<i>Figwort mosaic virus</i> (FMV) promoter and T-nos terminator from <i>A. tumefaciens</i>
<i>cry34Ab1</i>	Insecticidal δ -endotoxins Cry34Ab1 originating from <i>B. thuringiensis</i> strain PS149B1 ^{12,13,38}	Resistance to certain coleopteran insects	UbiZM1 promoter from <i>Zea mays</i> and PINII terminator from <i>Solanum tuberosum</i>
<i>cry35Ab1</i>	Insecticidal δ -endotoxins Cry35Ab1 originating from <i>B. thuringiensis</i> strain PS149B1 ^{12,13,38}	Resistance to certain coleopteran insects	TA peroxidase promoter from <i>T. aestivum</i> and PINII terminator from <i>S. tuberosum</i>
<i>cry1F</i>	A variant of the Cry1F protein originating from <i>B. thuringiensis</i> subsp. <i>aizawa</i> ^{9,10,39}	Resistance to certain lepidopteran insects	UbiZMI promoter from <i>Z. mays</i> and ORF25polyA terminator from <i>A. tumefaciens</i>
<i>cry3Bb1</i>	Variation of the Cry3Bb1 protein originating from <i>B. thuringiensis</i> subsp. <i>kumamotoensis</i> ^{11,40}	Resistance to certain coleopteran insects	PIIG (<i>Z. mays</i>) promoter and Hsp17 3'UTR from <i>T. aestivum</i>
dsRNA <i>DvSnf7</i>	Expresses a dsRNA transcript that contains a fragment of the <i>Snf7</i> gene ^{11,41}	Resistance to certain coleopteran insects	CaMV e35S promoter and E9 gene 3'UTR from <i>Pisum sativum</i>
<i>See references for a detailed description of the traits</i>			

3.3 Molecular characterisation

Previously, COGEM evaluated the molecular characterisation of each parental line and considered these to be adequate.^{7,8,9,10,11,12,13,14}

The applicant compared the inserted sequences in MON87427xMON89034x1507xMON87411x59122xDAS-40278-9 with the previously determined sequences for the single events. For 1507, the sequence derived from the breeding stack contains a single mutation in the *cry1F* gene compared to

the 1507 single event (reference) sequence. The breeding stack sequence also contains a single mutation in the 5' flanking region of the 59122 derived insert, compared to the single event (reference) sequence. The insert and flanking borders sequences from DAS-40278-9, MON87427, MON89034 and MON87411 in the breeding stack were identical to the single events. In application EFSA/GMO/NL/2017/144, regarding import and processing of GM maize MON87427xMON89034xMIR162xMON87411, a polyC tract downstream of the insertion site in the MON87427 event was mentioned. The exact length of this polyC tract could not be determined, therefore the applicant indicated 10 cytosine residues in the alignment to represent the polyC tract of MON87427 in the stacked line. COGEM notes that in the current application (EFSA/GMO/NL/2017/145), this issue is not addressed in the molecular characterization of MON87427 in MON87427xMON89034x1507x MON87411x59122xDAS-40278-9.

The applicant updated the bioinformatics analyses of the inherited inserted elements using recent databases, and the sequences spanning the insertion sites and 5' and 3' flanking regions. According to the applicant, the putative products of the open reading frames spanning the 5' and 3' junctions of the inserts did not generate any protein sequence similarity with known allergens, toxins or other biologically active proteins. COGEM notes that the applicant used the single event (reference) sequences for the bioinformatics analyses. In the case of the 1507 and 59122 inserts, which contain mutations in the stacked line relative to the single events, it is therefore unclear what the consequences are of these mutations. Therefore, in addition to the use of updated databases in bioinformatics analyses, COGEM is of the opinion that the analyses should have been performed with the updated sequences of 1507 and 59122. Hence, COGEM considers the molecular characterisation of maize MON87427xMON89034x1507xMON87411x59122xDAS-40278-9 incomplete.

Conclusion: The molecular characterisation of maize MON87427xMON89034x1507x MON87411x59122xDAS-40278-9 does not contain bioinformatics analyses on updated sequence information, and is therefore incomplete.

3.4 Phenotypic and agronomic characteristics

Previously, COGEM evaluated the phenotypic and agronomic characteristics of each parental line of MON87427xMON89034x1507xMON87411x59122xDAS-40278-9^{7,8,9,10,11,12,13,14}, and found no deviations influencing the outcome of the environmental risk assessment.

The applicant analysed the phenotypic and agronomic characteristics of MON87427xMON89034x1507xMON87411x59122xDAS-40278-9 and noted that most agronomic characteristics did not differ from those in the non-GM near-isogenic line. When differences were observed, they were within ranges considered to be normal for conventional commercial reference maize. The results of the phenotypic 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 MON87427xMON89034x1507xMON87411x59122xDAS-40278-9 allow the GM maize to survive or establish in the Dutch environment.

Conclusion: There are no indications that the introduced traits allow MON87427xMON89034x1507xMON87411x59122xDAS-40278-9 to survive in the Netherlands. MON87427xMON89034x1507xMON87411x59122xDAS-40278-9 does not have an increased potential for the establishment of 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, RIKILT carries out a food and/or feed assessment for Regulation (EC) 1829/2003 applications. The outcome of the assessment by other organisations (EFSA, RIKILT) was not known when this advice was completed.

5. Post-market environmental monitoring (PMEM)

The applicant supplied a general surveillance plan as part of the PMEM. COGEM has published several recommendations for further improvement of the general surveillance (GS) plan,^{42,43} but considers the current GS plan adequate for the import and processing of maize MON87427xMON89034x1507xMON87411x59122xDAS-40278-9.

6. Overall conclusion

There are no indications that expression of the introduced traits will alter the fitness of maize MON87427xMON89034x1507xMON87411x59122xDAS-40278-9 under natural conditions. COGEM is of the opinion that the bioinformatics analyses of maize MON87427xMON89034x1507xMON87411x59122xDAS-40278-9 are incomplete, and deems it necessary to perform the bioinformatics analyses with updated sequence information before a risk assessment can be made.

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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