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**KENMERK** CGM/181220-03  
**ONDERWERP** Advies import en verwerking van gg-maïs  
MON89034x1507xMIR162xNK603xDAS-40278-9

Geachte mevrouw Van Veldhoven,

Naar aanleiding van een vergunningaanvraag voor import en verwerking van genetisch gemodificeerde maïs MON89034x1507xMIR162xNK603xDAS-40278-9 (EFSA/GMO/NL/2018/151), 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 MON89034x1507xMIR162xNK603xDAS-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*, *cry1F* en *vip3Aa20* tot expressie, waardoor de plant resistent is tegen bepaalde plaaginsecten die behoren tot de vlinderachtigen. De kruisingslijn bevat ook een *pmi* gen. Dit gen wordt gebruikt als hulpmiddel bij het maken van gg-planten.

Verwildering van maïsplanten is in Nederland nooit waargenomen. Maïsplanten uit gemorst zaad (opslagplanten) worden hier nauwelijks aangetroffen. Bovendien komt de wilde verwant van maïs, teosinte niet in Nederland voor in maïsvelen of in de natuur, waardoor de ingebrachte sequenties zich niet naar andere soorten kunnen verspreiden.

De moleculaire karakterisering van MON89034x1507xMIR162xNK603xDAS-40278-9 voldoet aan de eisen van de COGEM. Er zijn geen redenen om aan te nemen dat expressie van de ingebrachte genen ervoor zorgt dat deze gg-maïs zou kunnen verwilderen.

Gezien het bovenstaande acht de COGEM de milieurisico's van de import en verwerking van de gg-maïs MON89034x1507xMIR162xNK603xDAS-40278-9, en subcombinaties 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. 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  
MON89034x1507xMIR162xNK603xDAS-40278-9  
and sub-combinations**

**COGEM advice CGM/181220-03**

- The present application (EFSA/GMO/NL/2018/151) concerns the authorisation for import and processing for use in feed and food of genetically modified (GM) maize MON89034x1507xMIR162xNK603xDAS-40278-9 and its sub-combinations;
- The stacked event maize MON89034x1507xMIR162xNK603xDAS-40278-9 was produced by conventional crossbreeding of the five GM parental maize lines;
- Previously, COGEM advised positively on the import and processing of all five parental lines;
  
- The GM maize expresses the genes *cry1A.105*, *cry2Ab2*, *cry1F*, *vip3Aa20*, *cp4 epsps*, *pat*, *aad-1* and *pmi*;
- It is resistant to certain lepidopteran insects, tolerant to 2,4 dichlorophenoxyacetic acid, glyphosate and glufosinate-ammonium containing herbicides, and able to use mannose as a carbon source;
  
- 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 maize fields or in nature and hybridisation of GM maize with other species is therefore not possible;
  
- The molecular characterisation of MON89034x1507xMIR162xNK603xDAS-40278-9 has been updated and meets the criteria of COGEM;
- The updated molecular characterisation does not provide indications for potential environmental risks;
  
- There are no indications that the introduced traits allow the stacked event maize MON89034x1507xMIR162xNK603xDAS-40278-9 to survive in the Netherlands;
- There are no indications that MON89034x1507xMIR162xNK603xDAS-40278-9 could establish feral populations in the Netherlands;
  
- COGEM is of the opinion that import and processing of maize MON89034x1507xMIR162xNK603xDAS-40278-9, and GM maize consisting of sub-combinations of its parental GM maize lines poses 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/2018/151) filed by Dow AgroSciences LLC concerns import and processing of MON89034x1507xMIR162xNK603xDAS-40278-9 maize and genetically modified (GM) maize consisting of its sub-combinations. The GM maize was produced by conventional crossbreeding of the five genetically modified (GM) parental maize lines. It expresses the *cp4 epsps*, *pat* and *aad-1* genes conferring tolerance to 2,4-D, glyphosate and glufosinate-ammonium containing herbicides, and the *cryIA.105*, *cry2Ab2*, *cry1F* and *vip3Aa20* genes resulting in resistance to certain lepidopteran insects. In addition, it expresses the *pmi* gene. As a result transformed plant cells are able to use mannose as a carbon source.

The parental lines, MON89034<sup>1</sup>, 1507<sup>2</sup>, MIR162<sup>3</sup> and NK603<sup>4</sup> and DAS-40278-9<sup>5</sup>, have all been authorised for import and processing for use in food and feed in the European Union.

## 2. Previous COGEM advice

COGEM previously advised positively on import and processing of all five parental lines: MON89034<sup>6</sup>, 1507<sup>7,8</sup>, MIR162<sup>9</sup>, NK603<sup>10</sup> and DAS-40278-9<sup>11\*</sup>. COGEM also advised positively on the import and processing of several stacked events including MON87427xMON89034x1507xMON88017x59122<sup>12</sup>, MON87427xMON89034x1507xMON87411x59122x DAS-40278-9<sup>13</sup>, MON89034x1507xMON88017x 59122xDAS-40278-9<sup>14</sup> and MON89034x1507x NK603<sup>15</sup>.

## 3. Environmental risk assessment

Potential environmental risks of the stacked event maize MON89034x1507xMIR162xNK603xDAS-40278-9 and of GM maize consisting of its sub-combinations 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,<sup>16,17</sup> 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 probably highly limited but cannot be excluded.<sup>18</sup>

Recently, the wild relative of maize, teosinte was detected in Spain<sup>19,20,21</sup> and France.<sup>22,23</sup> In these countries, it is present as a weed in some arable fields. In the Netherlands, teosinte is absent in maize fields or in nature.<sup>24</sup> Hybridisation of GM maize with other species cannot occur. Maize requires warm conditions in order to grow.<sup>18,25</sup> 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.<sup>18</sup> Maize does not tolerate prolonged cold and frost. In the

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\* In the 2011 COGEM advice, the maize line DAS-40278-9 was incorrectly named DAS-40728-9.

Netherlands, any volunteers emerging in or outside maize fields will be killed by frost at the onset of winter.<sup>24</sup>

Maize is very sensitive to weed competition.<sup>26</sup> During the long process of domestication, maize has lost the ability to persist in the wild.<sup>17</sup> A soil seed bank, small seeds, and an extended period of flowering and seed production are characteristics often observed in persistent weeds.<sup>27</sup> Maize lacks all these characteristics. After ripening, the seeds (the kernels) adhere to the cob and do not shatter naturally.<sup>18,28</sup> Consequently, seed dispersal is severely hampered.

During field observations in Austria some volunteers and maize plants were observed in non-agricultural habitats.<sup>29</sup> In the Netherlands, the appearance of volunteers is very rare, although maize plants occasionally have been observed outside agricultural fields.<sup>30</sup> COGEM is not aware of any reports of feral maize populations in the Netherlands or elsewhere in Europe.

1 **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 MON89034x1507xMIR162xNK603xDAS-40278-9 was created by conventional cross-breeding of the parental lines. For a description of the parental lines, see previous COGEM advices.<sup>6,7,8,9,10,11</sup> 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
<i>cry1A.105</i> (MON89034)	The Cry1A.105 protein is a chimeric protein with domains from different Cry1 proteins from <i>Bacillus thuringiensis</i> subsp. <i>kumamotoensis</i> <sup>31</sup>	Resistance to certain lepidopteran insects	Enhanced 35S ( <i>e35S</i> ) promoter from <i>Cauliflower mosaic virus</i> (CaMV) and T-Hsp17 terminator from <i>Triticum aestivum</i>
<i>cry2Ab2</i> (MON89034)	Variant of the Cry2Ab2 protein from <i>B. thuringiensis</i> subsp. <i>kurstaki</i> <sup>31</sup>	Resistance to certain lepidopteran insects	Promoter from <i>Figwort mosaic virus</i> (FMV) and nopaline synthase ( <i>nos</i> ) terminator from <i>Agrobacterium tumefaciens</i>
<i>cry1F</i> (1507)	A variant of the Cry1F protein originating from <i>B. thuringiensis</i> subsp. <i>aizawa</i> <sup>32</sup>	Resistance to certain lepidopteran insects	Polyubiquitin ( <i>ubiZM1</i> ) promoter and intron from <i>Zea mays</i> and ORF25 terminator from <i>A. tumefaciens</i> strain pTi15955

Introduced genes	Encoded proteins	Traits	Regulatory elements
<i>pat</i> (1507)	Variant of phosphinothricin N-acetyltransferase (PAT) originating from <i>Streptomyces viridochromogenes</i> strain Tü 494 <sup>33,34,35</sup>	Tolerance to glufosinate-ammonium containing herbicides	35S promoter and 35S terminator from CaMV
<i>vip3Aa20</i> (MIR162)	Variant of a native vegetative insecticidal protein (Vip) class A, subclass a, (Vip3Aa20) originating from <i>B. thuringiensis</i> strain AB88 <sup>36</sup>	Resistance against certain lepidopteran insects	Polyubiquitin promoter and intron (ZmUbiInt) from <i>Zea mays</i> and 35S terminator from CaMV
<i>pmi</i> , also known as <i>manA</i> (MIR162)	Phosphomannose isomerase (PMI) enzyme derived from <i>Escherichia coli</i> K12 <sup>37</sup>	Enables transformed plant cells to use mannose as a sole carbon source	Polyubiquitin promoter and intron (ZmUbiInt) from <i>Z. mays</i> and nopaline synthase ( <i>nos</i> ) terminator from <i>A. tumefaciens</i>
<i>cp4 epsps</i> (NK603)	5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) from <i>Agrobacterium</i> sp. strain CP4 <sup>38,39</sup>	Tolerance to glyphosate containing herbicides	<i>Ract 1</i> promoter from <i>Oryza sativa</i> and nopaline synthase ( <i>nos</i> ) terminator from <i>A. tumefaciens</i>
<i>aad-1</i> (DAS-40278-9)	Aryloxyalkanoate dioxygenase (AAD-1) enzyme originating from <i>Sphingobium herbicidovorans</i> <sup>40</sup>	Cleavage of several synthetic auxins and certain 'aryloxyphenoxypropionate' (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	Polyubiquitin promoter and intron (ZmUbiInt) and Zmper5 3'UTR terminator from <i>Z. mays</i>

### 3.3 Molecular characterisation

Previously, COGEM evaluated the molecular characterisation of each parental line and considered these to be adequate.<sup>6,8,9,10,11</sup>

The applicant compared the MON89034, 1507, MIR162, NK603 and DAS-40278-9 insert and flanking sequences in MON89034x1507xMIR162xNK603xDAS-40278-9 with the sequences of the corresponding single events. According to the applicant the MON89034, NK603 and DAS-40278-9 inserts and flanking DNA regions in the stacked event maize are identical to the DNA sequences determined previously for the respective single events. The sequence derived from the breeding stack contains a G to A single nucleotide mutation (at position 3848 bp) in the *cryIF* gene compared to the 1507 single event (reference) sequence.

For MIR162, sequencing of the cloned PCR products from Fragments A and C showed minor variability in a stretch of 13 cytosines (2172-2184 bp) located in the ZmUbiInt promoter. It should be noted that homopolymers longer than eight base pairs have been associated with slipped-strand mispairing in PCR amplification, resulting in sequence differences between PCR template and final PCR amplicon.<sup>41,42,43</sup> To further investigate the sequence variability, the applicant directly sequenced two independent genomic clones containing this homopolymeric region. Analysis of sequencing data obtained from these genomic clones showed no sequence change compared to the reference sequence. The applicant therefore concluded that no nucleotide changes were present in the MIR162 insert and flanking sequence present in the maize stack genomic DNA when compared to the previously determined MIR162 single event (reference) sequence.

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.

The applicant states that he also conducted a bioinformatics assessment for similarity to known and putative allergens and toxins of translated stop codon-bracketed frames in 1507 maize included the translated sequence variation arising from the single nucleotide mutation G-to-A at position 3848 (referred to as position 5904) in the *cryIF* gene. COGEM considers the performed bioinformatics analyses adequate.

The molecular characterisation was conducted according to the criteria previously laid down by COGEM.<sup>44</sup> The results from the updated molecular characterisation do not provide indications that the stacked event maize MON89034x1507xMIR162xNK603xDAS-40278-9 could pose a risk to the environment.

**Conclusion:** The molecular characterisation of the stacked event maize MON89034x1507xMIR162xNK603xDAS-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 MON89034x1507xMIR162xNK603xDAS-40278-9, and found no deviations influencing the outcome of the environmental risk assessment.

The applicant analysed the phenotypic and agronomic characteristics of MON89034x1507xMIR162xNK603xDAS-40278-9 and noted that these, except for the introduced traits, are not different to the non-GM near-isogenic control line, and are equivalent to the reference varieties, taking into account natural variation. The results of the phenotypic evaluation do not give reason to assume that the GM maize could pose an environmental risk.

COGEM notes that the GM maize is resistant to certain insect pests, which may increase its vigour if these pests are present. However, there are several characteristics which contribute to the absent invasiveness and persistence of maize. Examples of these characteristics are the structure of the corn cob (seed shed enclosed in husks), the poor dormancy of kernels and the frost-sensitivity of maize.<sup>25,45</sup> As persistence and invasiveness are controlled by so many other characteristics, there is no reason to assume that the introduced traits will allow the GM maize to establish in the Netherlands.

In conclusion, COGEM is of the opinion that there are no reasons to assume that the introduced traits in the stacked event maize MON89034x1507xMIR162xNK603xDAS-40278-9 allow the GM maize to survive or establish in the Dutch environment.

**Conclusion:** There are no indications that the introduced traits allow the stacked event maize MON89034x1507xMIR162xNK603xDAS-40278-9 to survive in the Netherlands. MON89034x1507xMIR162xNK603xDAS-40278-9 has no 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,<sup>46,47</sup> but considers the current GS plan adequate for the import and processing of the stacked event maize MON89034x1507xMIR162xNK603xDAS-40278-9.

## 6. Overall conclusion

There are no indications that expression of the introduced traits will alter the fitness of the stacked event maize MON89034x1507xMIR162xNK603xDAS-40278-9 under natural conditions. COGEM is of the opinion that import and processing of MON89034x1507xMIR162xNK603xDAS-40278-9 maize and GM maize consisting of sub-combinations of its parental GM maize lines 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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