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**KENMERK** CGM/180924-01  
**ONDERWERP** Advies import en verwerking van gg-maïs  
Bt11xMIR162xMIR604xMON89034x5307xGA21

Geachte mevrouw Van Veldhoven,

Naar aanleiding van een vergunningaanvraag voor import en verwerking van genetisch gemodificeerde maïs Bt11xMIR162xMIR604xMON89034x5307xGA21 (EFSA/GMO/DE/2018/149), ingediend door Syngenta, 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 Bt11xMIR162xMIR604xMON89034x5307xGA21 en alle subcombinaties daarvan. In deze gg-maïs komen de genen *pat* en *mepsps* tot expressie, waardoor de plant tolerant is voor bepaalde herbiciden. Ook komen de genen *cry1Ab*, *vip3Aa20*, *mcry3A*, *cry1A.105*, *cry2Ab2* en *ecry3.1Ab* tot expressie, waardoor de plant resistent is tegen verschillende plaaginsecten die behoren tot de vlinder- en keverachtigen. De hybride bevat ook drie *pmi* genen. Deze zorgen ervoor dat gg-plantencellen na transformatie gemakkelijk geselecteerd kunnen worden.

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 moleculaire karakterisering van Bt11xMIR162xMIR604xMON89034x5307xGA21 is voldoende. 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 Bt11xMIR162xMIR604xMON89034x5307xGA21, 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 Bt11xMIR162xMIR604xMON89034x5307xGA21 and sub-combinations**

### **COGEM advice CGM/180924-01**

- The present application (EFSA/GMO/DE/2018/149) concerns the authorisation for import and processing for use in feed and food of genetically modified (GM) maize Bt11xMIR162xMIR604xMON89034x5307xGA21 and its sub-combinations;
- Maize Bt11xMIR162xMIR604xMON89034x5307xGA21 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 molecular characterisation of Bt11xMIR162xMIR604xMON89034x5307xGA21 has been updated and is adequate;
- The updated bioinformatics analysis does not provide indications for potential environmental risks;
  
- The GM maize expresses the genes *cry1Ab*, *vip3Aa20*, *mcry3A*, *cry1A.105*, *cry2Ab2*, *ecry3.1Ab*, *pat*, *mepsps*, *pmi* and *mir604pmi*;
- It is resistant to several lepidopteran and coleopteran insects, tolerant to 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, wild relatives of maize are absent and hybridisation of maize with other species is therefore not possible;
  
- There are no indications that the introduced traits allow Bt11xMIR162xMIR604xMON89034x5307xGA21 to survive in the Netherlands;
- There are no indications that Bt11xMIR162xMIR604xMON89034x5307xGA21 could establish feral populations in the Netherlands;
  
- COGEM is of the opinion that import and processing of maize Bt11xMIR162xMIR604xMON89034x5307xGA21 and its sub-combinations 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/DE/2018/149) filed by Syngenta concerns import and processing of genetically modified (GM) maize Bt11xMIR162xMIR604xMON89034x5307xGA21 maize and its sub-combinations. The GM maize was produced by conventional crossbreeding of the six genetically modified (GM) parental maize lines. It expresses the *pat* and *mepsps* genes conferring tolerance to glyphosate and glufosinate-ammonium containing herbicides, and expresses the *cry1Ab*, *vip3Aa20*, *mcry3A*, *cry1A.105*, *cry2Ab2* and *ecry3.1Ab* genes resulting in resistance to several lepidopteran and coleopteran insects. In addition, it expresses three *pmi* genes, including *mir604pmi*. As a result, transformed plant cells are able to use mannose as a sole carbon source.

Parental lines Bt11<sup>1</sup>, MIR162<sup>2</sup>, MIR604<sup>3</sup>, MON89034<sup>4</sup> and GA21<sup>5</sup> have been authorised for import and processing for use in food and feed in the European Union. Several sub-combinations of stacked events have also been authorised for import and processing for use in food and feed in the European Union.<sup>(e.g. 6)</sup> The parental line 5307 has been assessed for import and processing for use in food and feed. In 2015 EFSA has issued an inconclusive scientific and overall opinion, because it could not conclude on the safety of the eCry3.1Ab protein due to an inadequate toxicity study provided.<sup>7</sup> Recently, EFSA assessed a supplementary 28-day toxicity study in mice on the eCry3.1Ab protein and concluded that the toxicity study did not show adverse effects. Taking into account the previous assessment and the new information EFSA concluded that maize 5307 is as safe and nutritious as its conventional counterpart in the scope of the application.<sup>8</sup>

## 2. Previous COGEM advice

COGEM has previously advised positively on import and processing of all six parental lines: Bt11<sup>9,10</sup>, MIR162<sup>11</sup>, MIR604<sup>12</sup>, MON89034<sup>13</sup>, 5307<sup>14</sup> and GA21<sup>15,16</sup>. COGEM also advised positively on the import and processing of several stacked events including Bt11xMIR162xMIR604xGA21<sup>17</sup>, Bt11xMIR162x1507xGA21<sup>18</sup> and Bt11xMIR162xMIR604x1507x5307xGA21<sup>19</sup>. The environmental risks of import and processing of these GM maize events were considered negligible.<sup>9,11,12,13,14,15,16,17,18,19</sup>

## 3. Environmental risk assessment

Potential environmental risks of Bt11xMIR162xMIR604xMON89034x5307xGA21 maize and 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>20,21</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>22</sup>

Recently, the wild relative of maize teosinte was reported as a weed in maize fields in Spain<sup>23,24,25</sup> and France.<sup>26,27</sup> 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.<sup>22,28</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>22</sup>

Maize is very sensitive to weed competition.<sup>29</sup> During the long process of domestication, maize has lost the ability to persist in the wild.<sup>21</sup> A soil seed bank, small seeds, and an extended period of flowering and seed production are characteristics often observed in persistent weeds.<sup>30</sup> Maize lacks all these characteristics. After ripening, the seeds (the kernels) adhere to the cob and do not shatter naturally.<sup>22,31</sup> Consequently, seed dispersal is severely hampered.

During field observations in Austria some volunteers and maize plants were observed in non-agricultural habitats.<sup>32</sup> In the Netherlands, the appearance of volunteers is very rare, although maize plants occasionally have been observed outside agricultural fields.<sup>33</sup> 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 Bt11xMIR162xMIR604xMON89034x5307xGA21 was created by conventional cross-breeding of the parental lines. For a detailed description of the parental lines, see previous COGEM advices.<sup>9,10,11,12,13,14,15,16</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>cry1Ab</i> (Bt11)	A variant of the Cry1Ab protein from <i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> <sup>34</sup>	Resistance to certain lepidopteran insects	35S promoter from <i>Cauliflower mosaic virus</i> (CaMV) and nopaline synthase (NOS) terminator from <i>Agrobacterium tumefaciens</i>
<i>pat</i> (Bt11)	Variant of phosphinothricin N-acetyltransferase (PAT) originating from <i>Streptomyces viridochromogenes</i> strain	Tolerance to glufosinate-ammonium containing herbicides	35S promoter from CaMV and NOS terminator from <i>A. tumefaciens</i>

Introduced genes	Encoded proteins	Traits	Regulatory elements
	Tü 494 <sup>35,36</sup>		
<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>37,38</sup>	Resistance against certain lepidopteran insects	ZmUbiInt promoter from <i>Zea mays</i> and 35S terminator (CaMV)
<i>mcry3A</i> (MIR604)	A variant of the Cry3A protein from <i>B. thuringiensis</i> subsp. <i>tenebrionis</i> <sup>37,39,40</sup>	Resistance against certain coleopteran insects	Metallothionein-like (MTL) promoter from <i>Z. mays</i> and NOS terminator from <i>A. tumefaciens</i>
<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>41</sup>	Resistance to certain lepidopteran insects	e35S promoter (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>41</sup>	Resistance to certain lepidopteran insects	Promoter from <i>Figwort mosaic virus</i> (FMV) and NOS terminator from <i>A. tumefaciens</i>
<i>ecry3.1Ab</i> (5307)	A chimera of a variant of the Cry3A protein from <i>B. thuringiensis</i> subsp. <i>tenebrionis</i> (mCry3A) <sup>37,39,40</sup> and the Cry1Ab protein from <i>B. thuringiensis</i> subsp. <i>kurstaki</i> <sup>37,42,43,44</sup>	Resistance against certain coleopteran insects	CMP promoter from <i>Cestrum yellow leaf curling virus</i> and NOS terminator from <i>A. tumefaciens</i>
<i>mepsps</i> (GA21)	Modified 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) originating from <i>Z. mays</i> <sup>45</sup>	Tolerance to glyphosate containing herbicides	Ract1 promoter from <i>Oryza sativa</i> and NOS terminator from <i>A. tumefaciens</i>
<i>pmi</i> , including <i>mir604pmi</i> ; three copies (MIR162, MIR604, 5307)	Phosphomannose isomerase (PMI) enzyme and variant MIR604 PMI derived from <i>Escherichia coli</i> strain K12 <sup>46</sup>	Enables transformed plant cells to use mannose as a sole carbon source	ZmUbiInt promoter from <i>Z. mays</i> and NOS terminator from <i>A. tumefaciens</i>

Introduced genes	Encoded proteins	Traits	Regulatory elements
<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.<sup>9,10,11,12,13,14,15,16</sup>

The applicant compared the Bt11, MIR162, MIR604, MON89034, 5307 and GA21 insert and flanking sequences in Bt11xMIR162xMIR604xMON89034x5307xGA21 with the sequences of the corresponding single events. According to the applicant, the Bt11, 5307, GA21 and MON89034 insert and flanking DNA sequences in Bt11xMIR162xMIR604xMON89034x5307xGA21 maize are identical to the DNA sequences determined previously for the respective single events. For MIR162, sequencing data suggests a cytosine deletion in a homopolymer (mononucleotide repeat) region in the ZmUbiInt promoter sequence (bp 6770-6782). For MIR604, sequencing data suggests an adenine insertion in a homopolymer region in the 5' genomic flanking sequence (bp 158-167). The applicant states 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>47,48,49</sup> In neither case does the applicant consider nucleotide alterations in MIR604 and MIR162 to be indicative of a single nucleotide polymorphism in the stacked product compared to the single event, but considers them a reflection of the technical challenges faced when sequencing these regions.

The applicant updated the bioinformatics analyses of the inherited inserted elements, and the sequences spanning the insertion sites and the 5' and 3' flanking regions using recent databases. COGEM notes that the applicant did not use the sequence of the breeding stack in all of the bioinformatics analyses of MIR162 and MIR604. The sequences derived of MIR604 and MIR162 from the breeding stack suggest that they both contain a nucleotide variation in a homopolymer region compared to the single event sequences. The number of nucleotides in homopolymer regions is often variable in nature. Homopolymeric DNA tracts and other repetitive sequences can give rise to slippage of the polymerase during replication, resulting in an expansion or contraction of the new DNA strand.<sup>48,50</sup> They are, because of slipped-strand mispairing in PCR amplification, technically challenging to sequence.<sup>47,48,49</sup> Therefore, the suggested nucleotide variations are possibly not indicative of single nucleotide polymorphisms in the stacked product compared to the single events.

COGEM is of the opinion that in the event that the addition or deletion of a nucleotide in these homopolymer regions had occurred, it is unlikely that this would have changed the outcome of the bioinformatics analyses. The suggested nucleotide variations in the breeding stack may not be indicative of nucleotide polymorphisms in the stacked product but are possibly due to the technical challenges faced when sequencing these homopolymeric DNA tracts. Therefore, COGEM considers the performed bioinformatics analyses using the single event sequences adequate.

According to the applicant, no essential endogenous genes were disrupted at the insertion sites, and the putative products of the open reading frames (ORFs) spanning the 5' and 3' junctions of

the inserts, did not show significant protein sequence similarity with known allergens, toxins or other biologically active proteins.

COGEM considers the molecular characterisation of maize Bt11xMIR162xMIR604xMON89034x5307xGA21 adequate. The results from the updated molecular characterisation do not provide indications that Bt11xMIR162xMIR604xMON89034x5307xGA21 could pose a risk to the environment.

**Conclusion:** The molecular characterisation of maize Bt11xMIR162xMIR604xMON89034x5307xGA21 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 Bt11xMIR162xMIR604xMON89034x5307xGA21<sup>9,10,11,12,13,14,15,16</sup>, and found no deviations influencing the outcome of the environmental risk assessment.

The applicant analysed the phenotypic and agronomic characteristics of Bt11xMIR162xMIR604xMON89034x5307xGA21 and noted that these, except for the introduced traits, are not different to those of its conventional counterpart, 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 GM maize is resistant to certain insect pests, which may affect 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>51,52</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 Bt11xMIR162xMIR604xMON89034x5307xGA21 allow the GM maize to survive or establish in the Dutch environment.

**Conclusion:** There are no indications that the introduced traits allow Bt11xMIR162xMIR604xMON89034x5307xGA21 to survive in the Netherlands.  
Bt11xMIR162xMIR604xMON89034x5307xGA21 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,<sup>53,54</sup> but considers the current GS plan adequate for the import and processing of maize Bt11xMIR162xMIR604xMON89034x5307xGA21.

### **6. Overall conclusion**

There are no indications that expression of the introduced traits will alter the fitness of maize Bt11xMIR162xMIR604xMON89034x5307xGA21 under natural conditions.

COGEM is of the opinion that import and processing of Bt11xMIR162xMIR604xMON89034x5307xGA21 maize and its sub-combinations pose 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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