Import and processing of herbicide tolerant genetically modified maize MON87419

COGEM advice CGM/170929-02

- The present application (EFSA-GMO-NL-2017-140) concerns the authorisation for import and processing for use in feed and food of genetically modified (GM) maize MON87419;

- Maize MON87419 was produced by *Agrobacterium tumefaciens* mediated transformation with two separate T-DNA cassettes. T-DNA I contains the *dmo* and *pat* expression cassettes and T-DNA II contains the *cp4 epsps* expression cassette;
- Subsequently, crossing, segregation, screening and selection were used to isolate those plants that contain the *dmo* and *pat* gene expression cassettes (T-DNA I) and do not contain the *cp4 epsps* expression cassette (T-DNA II);
- This resulted in the MON87419 maize GM line which expresses the *dmo* and *pat* genes conferring tolerance to dicamba 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;

- The molecular characterisation of maize MON87419 meets the criteria of COGEM;
- There are no indications that the introduced traits alter the fitness of maize MON87419 under natural conditions;
- There are no reasons to assume that the introduced traits will allow GM maize MON87419 to survive in the Dutch environment;

- COGEM is of the opinion that import and processing of maize MON87419 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-2017-140), filed by Monsanto Europe S.A./N.V., concerns import and processing of genetically modified (GM) maize MON87419, for use in feed and food. Maize MON87419 was produced by *Agrobacterium tumefaciens* mediated transformation and subsequent crossing, segregation, screening and selection. The GM maize line contains the *dmo* and *pat* genes, conferring tolerance to dicamba and glufosinate-ammonium containing herbicides.

2. Previous COGEM advices

COGEM did not previously advise on the import and processing of maize lines containing the *dmo* and *pat* genes. COGEM did advise positively on the import and processing of several maize lines containing the *pat* gene in combination with additional transgenic traits, such as insect resistance.[1,2,3,4] COGEM has not previously advised on the import and processing of maize lines containing the *dmo* gene, but has advised positively on soybean and cotton lines containing this gene, including lines harbouring additional transgenic traits.[5,6,7,8]

3. Environmental risk assessment

3.1 Aspects of the wild-type crop

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,[9,10] 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.[11]

Recently the wild relative of maize teosinte, has been reported as a weed in maize fields in Spain[12,13,14] and France.[15,16] 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.[11,17] 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.[11]

Maize is very sensitive to weed competition.[18] During the long process of domestication, maize has lost the ability to persist in the wild.[10] A soil seed bank, small seeds, and an extended period of flowering and seed production are characteristics often observed in persistent weeds.[19] Maize lacks all these characteristics. After ripening, the seeds (the kernels) adhere to the cob and do not shatter naturally.[11,20] Consequently, seed dispersal is severely hampered.

During field observations in Austria some volunteers and maize plants were observed in non-agricultural habitats.[21] In the Netherlands, the appearance of volunteers is very rare, although, maize plants occasionally have been observed outside agricultural fields.[22] 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 and traits

Maize MON87419 was produced by *A. tumefaciens* mediated transformation using two separate T-DNA cassettes. The first T-DNA, designated as T-DNA I, contains the *dmo* and *pat* expression cassettes. The second T-DNA designated as T-DNA II, contains the *cp4 epsps* expression cassette. During transformation both T-DNA cassettes were inserted into the maize genome. Subsequently, crossing, segregation, screening and selection were used to isolate those plants that contain the *dmo* and *pat* gene expression cassettes (T-DNA I) and do not contain the *cp4 epsps* expression cassette (T-DNA II). This resulted in the GM MON87419 maize line, which expresses the *dmo* and *pat* genes conferring tolerance to dicamba and glufosinate-ammonium containing herbicides.

Introduced genes	**Encoded proteins (enzymes)**	**Traits**
dmo	Two variants of the dicamba mono-oxygenase (DMO) enzyme from *Stenotrophomonas maltophilia.* Except for 7 or 12 additional amino acids (DMO+7 and DMO+12 respectively) derived from the CTP4 and an additional leucine at position two, the DMO protein variants are identical to the wild-type DMO protein of *S. maltophilia*.[6,23,24,25]	Tolerance to dicamba containing herbicides
pat	The phosphinothricin N-acetyltransferase (PAT) enzyme originating from *Streptomyces viridochromogenes* strain Tü 494[1,26,27,28]	Tolerance to glufosinate-ammonium containing herbicides
For a detailed description of the traits see references		

3.3 Molecular characterisation

Next Generation Sequencing and Junction Sequence Analysis (NGS/JSA) showed that MON87419 contains one copy of the insert at a single integration locus and demonstrated the absence of PV-ZMHT507801 backbone and T-DNA II sequences. The applicant assessed the organisation of the elements within the DNA insert and the adjacent genomic DNA with directed sequence analysis and confirmed that each genetic element within the T-DNA I insert is intact, with the exception of the border regions. A comparison with the conventional control revealed that 602 bp were deleted from the maize genome. According to the applicant BLAST analyses indicate that the deleted DNA is not part of or within ~1000 bp of a protein-coding gene and no function could be assigned to the deleted DNA. Bioinformatic analyses indicate that no endogenous open reading frames (ORFs) are present in the region that flanks the T-DNA insertion site in the maize genome. According to the applicant it is therefore unlikely that endogenous genes were disrupted at the MON87419 insertion site. The applicant screened the sequences spanning the insertion sites at the 5’ and 3’ flanking regions for open reading frames (ORFs) from stop to stop codon. The applicant states that the putative products of the ORFs spanning the 5’ and 3’ junctions of the inserts, did not generate any relevant sequence similarity with known allergens, toxins or other biologically active proteins. Also, according to the applicant the T-DNA insert in MON87419 does not encode amino acid sequences with similarity to known allergens, toxins or other biologically active proteins present in recent databases. The molecular characterisation was conducted according to the criteria previously laid down by COGEM.[29]

Conclusion: The molecular characterisation of maize MON87419 is adequate and no indications for potential environmental risks were identified.

3.4 Phenotypic and agronomic characteristics

The applicant analysed the phenotypic and agronomic characteristics of MON87419. The introduced traits do not give reason to assume that MON87419 has an altered survivability compared to non-GM conventional counterparts and commercial reference maize hybrids under natural conditions. Therefore, COGEM is of the opinion that there are no indications that the introduced traits allow maize MON87419 to survive or establish in the Dutch environment.

Conclusion: MON87419 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,[30,31] but considers the current GS plan adequate for import and processing of maize MON87419.

6. Overall conclusion

COGEM is of the opinion that import and processing of maize MON87419 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.

7. Additional remark

In order to assess the environmental risks of a GM crop it is important to know if the seeds can survive in the soil and can form a persistent seedbank. The formation of a persistent seedbank is an important characteristic of plants that are considered to be weeds.[32,33] Although not relevant for the environmental risk assessment of MON87419, since maize does not form a seedbank and that there is no reason to assume that the introduced traits will change this, COGEM wants to point out that the experiments that have been done to determine the germination rate of MON87419 are not adequate to test the formation of a persistent seedbank. The applicant has only tested the primary dormancy of MON87419 and states that there are no differences when compared to conventional maize. Information about all types of dormancy can however be important for the environmental risk assessment of other GM crops.

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