# Import and processing of genetically modified maize MON87403 with increased biomass

## COGEM advice CGM/151210-01

**Summary**

*The present application (EFSA/GMO/BE/2015/125) concerns import and processing for use in feed or food of genetically modified maize MON87403. Cultivation is not part of this application.*

*Maize MON87403 was generated by* Agrobacterium *mediated transformation of conventional maize and expresses the gene* ATHB17. *As a result, maize MON87403 has an increased ear biomass compared to conventional maize.*

*During its long domestication process, maize has lost its ability to survive in the wild. In the Netherlands, the appearance of maize volunteers is rare and establishment of volunteers in the wild has never been reported. There are no reasons to assume that the introduced trait will allow maize MON87403 to establish feral populations. The introduced sequences cannot introgress into other species since wild relatives of maize are not present in Europe. COGEM is of the opinion that incidental spillage of MON87403 poses a negligible risk to the environment.*

*The molecular characterisation of MON87403 maize meets the criteria of COGEM.*

*In conclusion, COGEM considers the environmental risks associated with import and processing of maize MON87403 to be negligible. COGEM abstains from giving advice on the potential risks of incidental consumption because a food/feed assessment is already carried out by other organisations.*

**Introduction**

The scope of the present notification by Monsanto Europe S.A. concerns import and processing of genetically modified (GM) maize MON87403 for use in feed or food. It was produced by *Agrobacterium tumefaciens* mediated transformation of maize line LH244 and expresses the *ATHB17* gene. As a result, maize MON87403 has an increased ear biomass.

**Previous COGEM advice**

COGEM has not advised previously on import and processing of MON87403 maize or crops with an increased biomass.

**Aspects of the crop**

Maize (*Zea mays*) is a member of the grass family *Poaceae*. Maize is a highly domesticated crop, originating from Central America, but nowadays maize is cultivated globally. Maize is predominantly wind pollinated.[1,2] Insect pollination is limited since the female flowers do not produce nectar and are therefore not attractive to insect pollinators.[3] In Europe, no wild relatives of maize are present and therefore hybridisation with other species cannot occur.

Domesticated maize requires warm conditions in order to grow and does not tolerate prolonged cold and frost.[3,4] The seeds (kernels) remain on the cob after ripening and do not shatter naturally.[3,5] 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.[3]

Maize is very sensitive to weed competition.[6] During the long process of domestication, maize has lost the ability to survive in the wild.[2] In the Netherlands, the appearance of volunteers is very rare to absent.[7] Establishment of feral maize populations has never been observed in the Netherlands and COGEM is not aware of any reports of wild maize plants elsewhere in Europe.

**Molecular characterisation**

Maize MON87403 was produced by *A. tumefaciens* mediated transformation of maize line LH244 with plasmid PV-ZMAP5714. The T-DNA contains the *ATHB17* expression cassette.

An overview of the T-DNA introduced in MON87403 is given below:

- T-DNA Right border region of *A. tumefaciens*
- E35/Ract1: Chimeric promoter consisting of the duplicated enhancer region from the *Cauliflower mosaic virus* 35S RNA promoter combined with the promoter of the *act1* gene from *Oryza sativa*
- *Cab*: 5' untranslated region (UTR) leader sequence from the chlorophyll a/b-binding (CAB) protein of *Triticum aestivum*
- *Ract1*: Intron and flanking UTR sequence of the *act1* gene from *Oryza sativa*
- *ATHB17*: Coding sequence from the *ATHB17* gene from *Arabidopsis thaliana*
- *Hsp17*: 3'UTR sequence from the heat shock protein Hps17 of *Triticum aestivum*
- B-Left border region of *A. tumefaciens.*

Characterisation of the number of T-DNA insertions in MON87403 was conducted using a combination of Next Generation Sequencing (NGS) and Junction Sequence Analysis (JSA).[8,9] In addition, directed sequencing (locus specific PCR on non-fragmented genomic DNA and sequence analyses) was used to verify the sequences of the DNA insert and the maize genomic DNA flanking the insert.

For NGS, short (approximately 100 bp) randomly distributed sequenced fragments (sequencing reads) were generated. To determine the average coverage of the genome, all reads that mapped to a known endogenous single copy gene (*Pyruvate decarboxylase*, *pdc3*) were analysed. The median number of times any base of this gene is independently sequenced was used to determine the average coverage of the genome. According to the applicant, the analysis showed that *pdc3* was covered >110x for each sample.

NGS and JSA were used to determine the presence of backbone and T-DNA sequences, and the T-DNA copy number. Based on this analysis, the applicant concludes that MON87403 maize contains one copy of the T-DNA insert at a single integration locus and that no backbone sequences of plasmid PV-ZMAP5714 were inserted in the maize genome. The applicant performed this analysis on multiple generations and concludes that the insert is stably inserted.

By 'directed sequencing', the applicant determined the sequence of the insert and the flanking DNA. The applicant concludes that the inserted T-DNA is identical to the T-DNA of plasmid PV-ZMAP5714 with the exception of small terminal deletions at the Left and Right Border regions. There was also a 149 bp deletion of genomic DNA at the insertion site. BLASTn and BLASTx analyses were performed to determine if the insertion of the T-DNA had disrupted any endogenous genes located in the maize genomic DNA flanking the insert. According to the applicant, these analyses indicated that it is unlikely that endogenous genes at the insertion site were disrupted.

The applicant analysed the junctions between the insert and the maize genome sequence from stop codon to stop codon for the presence of potential novel open reading frames (ORFs). A total of 10 ORFs encoding putative proteins of 8 amino acids or greater were identified and evaluated for potential sequence similarities to known allergens, toxins and biologically active proteins. The applicant found no significant similarities.

The applicant states that bioinformatical analysis on the putative peptides encoded by the T-DNA insert did not demonstrate similarities to known allergens, toxins and biologically active proteins, apart from the introduced *ATHB17*gene.

In summary, MON87403 maize contains a single intact T-DNA insert. At the insertion site 149 bp are deleted. No endogenous gene is disrupted and no backbone sequences are present in the plant genome. Bioinformatic analysis of ORFs spanning the insert-genome junctions and within the insert identified no significant amino acid sequence similarities to known allergens, toxins and biologically active proteins. The molecular characterization of maize MON87403 meets the criteria laid down by COGEM.[10]

***ATHB17***

MON87403 contains the *ATHB17* gene that is derived from *A. thaliana*. In *A. thaliana* ATHB17 is expressed in the root quiescent center.[11,12] ATHB17 belongs to the family of HD-Zip class II proteins. HD-Zip class II proteins mediate the response of plants to a range of environmental conditions (for instance light quality and intensity) and regulate plant development.[11] These proteins can form homo- or heterodimers with members of their subfamily and as dimers they can bind target DNA. In Arabidopsis ATHB17 regulates leaf morphology and photosynthesis (chlorophyll content and the number and size of chloroplasts).[11] In *A. thaliana* overexpression of ATHB17 results in enhanced chlorophyll content and improved photosynthetic capacity.[11]

In maize a gene closely related to *ATHB17* has been identified, *Zmhdz18*.[13] This protein is predominantly expressed in reproductive tissues (ear inflorescence and cob tissue) but not in the

kernels or the whole ear, suggesting its involvement in regulation of plant reproductive development.[14]

The introduction of *ATHB17* in MON 87403 results in maize-specific splicing of the mRNA and a truncated protein, ATHB17Δ113. The ATHB17Δ113 protein retains the ability to bind to target DNA sequences. ATHB17Δ113 is, however, unable to function as a transcriptional repressor because the protein lacks a functional repression domain. Because of this condition, expression of ATHB17Δ113 leads to an increased ear weight in an early reproductive stage.[14]

**Food/ feed assessment**

COGEM abstains from giving advice on the potential risks of incidental consumption since a food/feed assessment is already carried out by other organisations.[15] 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/feed assessment for Regulation (EC) 1829/2003 applications is carried out by RIKILT. The outcome of the assessment by these organisations (EFSA, RIKILT) was not known upon completion of this advice.

**Considerations and advice**

COGEM has been asked to advice on import and processing of GM maize line MON87403. Maize MON87403 was generated by *Agrobacterium* mediated transformation of conventional maize and expresses the *ATHB17* gene. As a result, maize MON87403 has an increased ear biomass.

In case of incidental spillage, maize kernels may be released into the environment. However, volunteers are rarely found in Europe and establishment of feral populations has never occurred.[16] Maize has lost the ability to survive in the wild during the long process of domestication.[16] The biological characteristics of maize determine its disability to establish feral populations under the European climatic conditions.[17] Some of the characteristics of maize which determine the absence of invasiveness and persistence is given below:

- due to the structure of the corn crop (seed shed enclosed in husks) kernels remain on the cob after ripening[16]
- the kernels exhibit poor dormancy[16]
- maize is naturally frost-sensitive[17]
- maize is sensitive to weed competition[16]

COGEM is of the opinion that the risk of spread of MON87403 maize within the Netherlands due to incidental spillage of MON87403 is negligible. In MON87403 a gene is introduced which leads to production of larger cobs. In the opinion of COGEM there is no reason to assume that the introduced trait influences the ability of the GM maize to survive in the wild, since persistence and invasiveness are controlled by many other traits. Moreover, in field trials no significant differences were observed in biological characteristics determining invasiveness and persistence when comparing GM maize MON87403 with conventional maize. Introgression of the introduced

sequences into closely related species cannot occur, as wild relatives of maize are not present in Europe.[16]

The molecular characterisation of MON87403 maize meets the criteria of COGEM. COGEM has published several recommendations for further improvement of the standard general surveillance (GS) plan [18,19] but considers the submitted GS plan in the application adequate for import and processing of MON87403.

In view of the above, COGEM is of the opinion that import and processing of maize line MON87403 poses a negligible risk to the environment.

**Additional remark**

The Post-Market Environmental Monitoring (PMEM) plan for import and processing of MON87403 maize contains URLs for operators and importers/traders, which provides overviews of approved genetically modified plant products and the locations where the general surveillance should be carried out. COGEM notes that the URLs are no longer functional. Apparently the URLs are outdated.

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