Import and processing of herbicide tolerant genetically modified maize VCO-01981-5

COGEM advice CGM/160711-01

- The present application (EFSA/GMO/DE/2016/130) concerns import and processing for use in feed and food of genetically modified maize VCO-01981-5;
- Maize VCO-01981-5 was produced by *Agrobacterium tumefaciens* mediated transformation of hybrid maize line Hi-II with plasmid pAG3541;
- The GM line expresses the *epsps grg23ace5* gene conferring tolerance to glyphosate containing herbicides;
- In the Netherlands feral maize populations cannot occur;
- Hybridisation of maize with other species is not possible in the Netherlands;
- There are no indications that expression of the introduced trait will alter the fitness of VCO-01981-5 maize;
- The molecular characterization of VCO-01981-5 maize is adequate, but COGEM observed artefacts in some of the Southern blot figures. Therefore, it urges EFSA to retrieve and appraise the original data and figures in order to exclude the possibility of data manipulation.
- Provided that the authenticity of the Southern blot figures can be verified, COGEM is of the opinion that import and processing of maize VCO-01981-5 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 already carried out by other organisations.

1. Introduction

The scope of the present application (EFSA/GMO/DE/2016/130) filed by Genective S.A., is import and processing of genetically modified (GM) maize VCO-01981-5 for use in feed and food. VCO-01981-5 was produced by *Agrobacterium tumefaciens* mediated transformation of hybrid line Hi-II and expresses the *epsps grg23ace5* gene. This gene encodes an EPSPS protein called EPSPS ACE5 with low binding affinity to glyphosate. As a result, VCO-01981-5 is tolerant to glyphosate containing herbicides.

Previous COGEM advices

COGEM has not yet advised on import and processing of VCO-01981-5 maize or other events expressing the *epsps grg23ace5* gene. COGEM has advised positively on import and processing of several maize events genetically modified to express *cp4 epsps* genes.[1,2,3]

1. Environmental risk assessment

1.1 Aspects of the wild-type 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.[4,5] Insect pollination is limited since the female flowers do not produce nectar and therefore are not attractive to insect pollinators.[6] In Europe, no wild relatives of maize are present and so hybridisation with other species cannot occur.

In the Netherlands, the appearance of volunteers is very rare to absent.[7] Domesticated maize requires warm conditions in order to grow and does not tolerate prolonged cold and frost.[6,8] The kernels remain on the cob after ripening and do not shatter naturally.[6,9] 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.[6]

Maize is very sensitive to weed competition.[10] During the long process of domestication, maize has lost the ability to survive in the wild.[5] Establishment of maize plants in the wild has never been observed in the Netherlands and COGEM is not aware of any reports of feral maize populations elsewhere in Europe.

Conclusion: In the Netherlands feral maize populations cannot occur. Hybridisation of maize with other species is not possible in the Netherlands.

2.2 Description of the introduced genes and traits

Maize VCO-01981-5 was produced by *A. tumefaciens* mediated transformation of hybrid line Hi-II with plasmid pAG3541. The T-DNA contains the *epsps grg23ace5* expression cassette. The plasmid backbone contains, amongst other things, the *aadA* antibiotic resistance gene, which is used as a selection marker.

Introduced genes	Encoded protein	Trait
epsps grg23ace5	EPSPS ACE5. Optimized form of the native 5-enolpyruvulshikimate-3-phosphate synthase (EPSPS GRG23) enzyme originating from *Arthrobacter globiformis*	Tolerance to glyphosate containing herbicides, because of a decreased binding affinity for glyphosate.
For a detailed description of the trait (resulting from the expression of another *epsps* gene) see reference 11.		

2.3 Molecular characterization

Southern blot analyses showed that maize VCO-01981-5 contains one copy of the insert at a single integration locus. Southern blot analyses also demonstrated the absence of backbone sequences. The applicant determined the sequence of the insert and adjacent flanking genomic DNA

sequences. A 22 bp deletion on the right border and a 16 bp deletion on the left border of the inserted T-DNA were identified compared to the original sequence in plasmid pAG3541. A comparison with the parental maize line revealed that a 21 bp deletion was created in the maize genome by the T-DNA insertion.

The T-DNA was inserted in the promoter or in the 5' untranslated region of an Acanthoscurrin homologous gene. Regular transcription of this Acanthoscurrin like gene is probably prevented by the insertion of the T-DNA. Since Acanthoscurrin is an antimicrobial peptide, blocking transcription may result in increased disease susceptibility. However, no phenotypic differences in disease susceptibility or pest resistance were observed in maize VCO-01981-5 relative to its conventional counterpart.

The applicant screened the junctions between the T-DNA insert and the flanking plant genomic DNA for potential newly created open reading frames (ORFs). Twelve ORFs potentially encoding peptides of eight amino acids or more were identified at the T-DNA junctions. In addition, 234 ORFs potentially encoding peptides of eight amino acids or more were identified inside the T-DNA. The putative amino acid sequences were compared to a dataset of known and putative allergen sequences (Allergen Online, version 15) and to an Internal Limagrain database to identify possible similarities to known toxins. The applicant states that no similarities to toxins were identified. Two ORFs showed low similarities to an allergen. However, the sequences producing these matches ('hits') are not part of the insert. These matches are part of the endogenous maize genome and also present in the conventional counterpart. Furthermore, it is unlikely that the positive hit represents an actual allergen, since necessary elements (like epitopes, domains and glycosylation signals) appear to be lacking.

Conclusion: The molecular characterization of VCO-01981-5 maize is adequate. There are no indications that expression of the introduced trait will alter the fitness of VCO-01981-5 maize.

COGEM notes that in several of the Southern blot figures there appear to be halos around some of the bands (for instance in 'Part II – Scientific Information' figure 17, and the left panels of figures 18, 19 and 23). These halos are visible in the print-outs, but are more difficult to identify in the digital file. These could be artefacts due to the conversion of the original figures into files of jpeg or pdf format, or caused by smoothening or sharpening during image processing. However, the exact cause or nature of these halos cannot be determined from the pdf's made available to COGEM. Therefore, COGEM urges EFSA to appraise the original data and figures to exclude the possibility of data manipulation. If the authenticity of the figures cannot be verified, the conclusions of COGEM concerning the Southern blot analyses and, consequently, the environmental risk assessment have to be considered void.

2.4 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, a food and/or feed assessment for Regulation (EC) 1829/2003 applications is carried out by RIKILT. The outcome of the assessment by other organisations (EFSA, RIKILT) was not known when this advice was completed.

3. General surveillance

COGEM has published several recommendations for further improvement of the general surveillance (GS) plan[12,13] but considers the current GS plan adequate for import and processing of VCO-01981-5 maize.

4. Overall conclusion

Provided that the authenticity of the Southern blot figures can be verified, COGEM is of the opinion that import and processing of maize VCO-01981-5 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 already carry out a food/feed assessment.

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