Renewal of the authorisation for import and processing of genetically modified 59122 maize

COGEM advice CGM/161129-01

- The present application (EFSA/GMO/RX/003) concerns the renewal of the authorisation for import and processing for use in feed and food of genetically modified 59122 maize;
- Maize 59122 has previously been authorized for import and processing in 2007;
- In 2005, COGEM has advised positively on the import and processing of 59122;
- Maize 59122 expresses the *cry34Ab1* and *cry35Ab1* genes conferring resistance to certain coleopteran insects, and the *pat* gene conferring tolerance to glufosinate-ammonium containing herbicides;
- In the Netherlands, feral maize populations have never been observed and the appearance of volunteers is rare to absent;
- In the Netherlands, wild relatives of maize have never been observed and hybridisation of maize with other species is not possible;
- The molecular characterisation and bioinformatic analyses of 59122 maize have been updated and meet the criteria of COGEM;
- There are no indications that the introduced traits will alter the fitness of 59122 maize;
- The updated molecular characterisation, literature review and monitoring reports do not give any indication of a potential environmental risk;
- COGEM is of the opinion that import and processing of 59122 maize 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/RX/003), filed by Pioneer Hi-Bred Int. and Dow AgroScience LLC, concerns the renewal of the consent for import and processing of the genetically modified (GM) 59122 maize (unique identifier DAS-59122-7) for use in feed and food. Maize 59122 contains the *cry34Ab1*, *cry35Ab1* and *pat* genes, conferring insect resistance and herbicide tolerance to the crop. Maize 59122 has been authorised for import, food, feed and processing in Europe on October 24th in 2007 (2007/702/EC). Since import and processing authorisations remain valid for a period of 10 years, the applicant filed an application for the renewal of the authorisation for import and processing. The application contains amongst others monitoring reports, an updated molecular characterisation and an updated literature search.

2. Previous COGEM advices

COGEM has advised positively on the import and processing of 59122 maize in 2005.[1] COGEM has also repeatedly advised positively on the import and processing of stacked maize lines derived from the 59122 parental line, e.g. maize Bt11x59122xMIR604x1507xGA21[2] and 1507x59122x MON810xNK603[3].

COGEM has also advised on the cultivation of 59122 maize. Cultivation of 59122 maize was deemed of negligible risk to the environment by COGEM in 2008[4] and 2013[5] (following the publication of a new EFSA opinion), but only under the condition that 'case specific monitoring' of ladybird beetles is conducted. In 2013 EFSA issued an opinion[6], which stated that additional laboratory studies on ladybird beetles and honeybees are required prior to authorization of 59122 maize cultivation. In 2008, COGEM advised positively on cultivation of the stacked maize line 1507x59122[7], again under the condition of 'case specific monitoring' of ladybird beetles.

3. Environmental risk assessment

3.1 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 it is cultivated globally. Maize is predominantly wind pollinated.[8,9] Insect pollination is limited since maize has both male and female flowers that are spatially separated. Insects do not visit the female flowers because they do not produce nectar or pollen and are therefore not attractive to insect pollinators.[10]

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.[10,11] After ripening, the kernels remain on the cob and do not shatter naturally.[10,12] 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.[10] In the Netherlands, the appearance of volunteers is very rare to absent.[13]

Maize is very sensitive to weed competition.[14] During the long process of domestication, maize has lost the ability to survive in the wild.[9] 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 do not occur and hybridisation of maize with other species is not possible.

3.2 Description of the introduced genes and traits

Maize 59122 is produced by means of *Agrobacterium*-mediated transformation. This maize line contains the genes *cry34Ab1* and *cry35Ab1* isolated from the bacterium *Bacillus thuringiensis* (*Bt*) to provide protection against coleopteran pests, such as the western corn rootworm (*Diabrotica virgifera virgifera*). The genes encode the proteins Cry34Ab1 and Cry35Ab1, which act together to

provide maximum protection against corn rootworm pests by affecting the midgut epithelium after ingestion and causing death.[15,16,17] Maize 59122 also contains the *pat* gene, isolated from the bacterium *Streptomyces viridochromogenes*, which expresses the PAT protein that confers tolerance to glufosinate-ammonium containing herbicides.[18] The PAT enzyme acetylates L-phosphinothricin (L-PPT), the active ingredient in glufosinate-ammonium, which is thereby reduced to an inactive form. Inactivation of L-PPT prevents binding to glutamine synthetase and enables the synthesis of L-glutamine required for the detoxification of excess ammonia.[19]

Introduced genes	**Encoded proteins**	**Traits**
cry34Ab1, *cry35Ab1*	Insecticidal δ-endotoxins Cry34Ab1 and Cry35Ab1[20,21]	Insect resistance against coleopteran pest species.
pat	Phosphinothricin N-acetyltransferase (PAT) enzyme [19,20,21]	Tolerance to glufosinate-ammonium herbicides.
For a detailed description of the introduced genes and traits, see for example references 19, 20 and 21		

3.3 Updated molecular characterisation

The application for the renewal of the authorisation for import and processing of 59122 maize contains an updated molecular characterisation carried out using recent databases, which meet the requirements of COGEM.[22]

The 59122 insert and flanking regions in the maize genome were originally sequenced in 2002. The 2002 sequence analyses revealed 2 base pair changes in the insert sequence compared to the plasmid T-DNA. Re-sequencing in 2016 revealed 2 base pair changes relative to the insert sequence from 2002, but matching the original plasmid T-DNA sequence. According to the applicant, re-sequencing of DNA from the original 59122 maize material confirmed that the original sequence deviations reported in 2002 were misreads.[*] The base pair changes are not located in the sequences coding for the Cry34Ab1, Cry35Ab1 and PAT proteins.

The molecular characterisation performed in 2016 confirmed that no endogenous maize genes were disrupted by the T-DNA insertion. The entire T-DNA insert and its flanking regions were screened for putative stop-to-stop open reading frames (ORFs). The ORFs were defined as sequences between two stop codons with a coding capacity of minimal eight amino acids. Ten across-junction ORFs, and 433 ORFs within the T-DNA insert were identified and evaluated for encoding potential protein sequences with similarity with known proteins. No similarities to known toxins were identified. The applicant reports sequence homology between the Cry34Ab1 protein and aegerolysin domain-containing proteins. This is expected, since Cry proteins have been identified as aegerolysin-like proteins in the literature.[23] Aegerolysins are proteins that are capable of inducing pores in lipid membranes with other pore-forming proteins. Some aegerolysins, e.g., those produced by certain fungi, can have hemolytic properties.[24] However, as applicant states, the aegerolysin domain of Cry34Ab1 has no conserved amino acids related to hemolytic properties.

[*] Another misread was identified in the 5' flanking region. As the base pair change is positioned outside the insert in the maize genomic DNA, it is not considered relevant to the environmental risk assessment.

Conclusion: The molecular characterisation of 59122 maize is adequate and no indications for potential environmental risks were identified.

3.4 Annual monitoring reports

The applicant supplied annual monitoring reports carried out between December 2008 and October 2014. Monitoring was performed by operators involved in the import, handling and processing of 59122 maize; i.e., COCERAL, UNISTOCK and FEDIOL. Also, as part of the monitoring, the applicant performed a yearly review of scientific publications to monitor the safety of 59122 maize. The operators did not perform monitoring in 2015. According to the applicant 59122 maize has not been commercialised as a single product for some time now, and it is not available for import to the European Union. Only the systematic search and review of studies published in the scientific literature was performed in 2015. The monitoring reports and the scientific publications contained no reports on adverse effects or incidents.

Conclusion: Annual monitoring reports give no indication of adverse effects or incidents resulting from import and/or processing of 59122 maize.

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. General surveillance

The applicant supplied a new post-market environmental monitoring (PMEM) plan. COGEM has published several recommendations for further improvement of the general surveillance (GS) plan[25,26] but considers the current GS plan adequate for import and processing of 59122 maize.

In its advice on the application for cultivation of 59122 maize, COGEM expressed concerns with regard to ladybird beetles. EFSA later expressed additional concerns about honeybees. COGEM noted that the applicant did not address these concerns in the application. However, as this application entails import and processing of 59122 maize, COGEM considers the current approach acceptable.

6. Overall conclusion

There are no indications that expression of the introduced traits will alter the fitness of 59122 maize. Import and processing of 59122 maize poses a negligible risk to the environment in the Netherlands. COGEM abstains from giving advice on the potential risks of incidental consumption since other organizations carry out a food/feed assessment.

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