Advice on the import and processing of GM maize MZIR260

COGEM advice CGM/251105-01

- The present application (GMFF-2025-22144) concerns the authorisation for import and processing for use in food and feed of genetically modified (GM) maize MZIR260;
- Maize MZIR260 was produced by *Agrobacterium*-mediated transformation, and produces the eCry1Gb.1Ig protein, a chimera of the Cry1Gb and Cry1Ig proteins, which confers resistance to certain susceptible lepidopteran insects;
- MZIR260 also produces the enzyme phosphomannose isomerase (PMI). PMI was used as a selectable marker in the development of MZIR260 maize and enables the plant cells to utilize mannose as a primary carbon source;

- In the Netherlands, feral maize populations have never been observed and the appearance of volunteers – maize not deliberately planted - is rare;
- In the Netherlands, the wild relative of maize, teosinte, is not present in the natural environment, hybridization of GM maize with other species is therefore impossible;

- The molecular characterisation of maize MZIR260 meets the criteria of COGEM, and no indications for potential environmental risks were identified;
- There are no indications that the introduced traits will allow GM maize MZIR260 to survive in the Dutch environment;

- COGEM is of the opinion that import and processing of maize MZIR260 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 conduct a food/feed assessment.

1. Introduction

The present application (GMFF-2025-22144), filed by Syngenta Crop Protection NV/SA, acting on behalf of Syngenta Crop Protection AG, concerns the import and processing of genetically modified (GM) maize MZIR260. MZIR260 carries the *eCry1Gb.1Ig-03* gene, which encodes the engineered eCry1Gb.1Ig protein, a chimera of the Cry1Gb and Cry1Ig proteins. Both Cry1Gb and Cry1Ig are active against several lepidopteran pest species. MZIR260 also carries the gene *pmi-15* (also known as *manA*), which encodes the enzyme phosphomannose isomerase (PMI). PMI enables transformed plant cells to use mannose as a sole carbon source and was used as a selectable marker in the development of MZIR260 maize.

2. Previous COGEM advice

COGEM has not previously advised on GM maize that expresses the chimeric *eCry1Gb.1Ig-03* gene introduced in maize MZIR260, but has issued positive opinions on GM maize that is resistant to certain lepidopteran pests due to the expression of (chimeric) Cry proteins,[1,2,3,4,5,6] including GM maize combining Cry protein and PMI expression.[7,8,9,10,11,12]

3. Environmental risk assessment

The objective of an environmental risk assessment (ERA) is to identify and evaluate potential adverse effects of the genetically modified organism (GMO), direct or indirect, immediate, or delayed, on human health and the environment. This ERA involves the import and processing of GM maize. Any concerns relating to cultivation, management or harvesting practices are beyond the scope of this advice. When assessing the environmental risk of incidental spillage of GM maize COGEM first considers the likelihood that the event could establish itself in the Netherlands or could hybridise with related species. Other so-called 'areas of concern' (e.g. effects on non-target organisms) are addressed only if there is a possibility that the event could establish itself or if gene flow to other species might occur.

3.1 Characteristics of the crop

Maize (*Zea mays*) is a member of the grass family *Poaceae*. It is a highly domesticated crop that originated in Central America and is nowadays cultivated globally. Maize is wind-pollinated[13,14] 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 highly limited but cannot be excluded.[15] There are no known instances of hybridisation between GM maize and species other than teosinte, the wild relative of maize.

Maize does not tolerate prolonged cold or frost and requires warm conditions to grow.[15,16,17] In cultivation areas with warm climatic conditions, volunteers (maize not deliberately planted) can be present 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.[15]

Maize is very sensitive to weed competition.[18] During the long process of domestication, maize has lost the ability to persist in the wild.[14] 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 scatter naturally.[15,20] Consequently, seed dispersal is severely hampered.

3.2 Receiving environment

In the Netherlands, the appearance of volunteers is rare, although maize plants have occasionally been observed outside agricultural fields.[21,22] Any volunteers that emerge will be killed by frost at the onset of winter.[22] COGEM is not aware of any reports of feral maize populations in the Netherlands.[22]

Maize can hybridise with teosinte, the wild relative of maize. However, as teosinte is absent from maize fields and nature in the Netherlands,[22] hybridisation of GM maize with teosinte will not occur in the Netherlands.

Conclusion: In the Netherlands, feral maize populations do not occur. Therefore, hybridisation of GM maize with other species is impossible.

3.3 Description of the introduced genes and traits

GM maize MZIR260 was produced by *Agrobacterium tumefaciens*-mediated transformation of immature maize embryos from maize line AX5707, using plasmid pSYN24795. This plasmid vector contains a single transfer DNA (T-DNA), which comprises the gene cassettes *eCry1Gb.1Ig-03* and *pmi-15*.

Crystal (Cry) proteins, naturally produced by the bacterium *Bacillus thuringiensis*, consist of three domains and are known for their insecticidal activity against various lepidopteran insects. As a result, they are commonly used as insecticidal traits in GM crops such as GM soybean and GM maize.[23]

The chimeric protein eCry1Gb.1Ig expressed in GM maize MZIR260 comprises the first two domains from Cry1Gb, the third domain from a Cry1Ig-like protein, and the protoxin tail of Cry1Gb. eCry1Gb.1Ig was engineered to be effective against fall armyworm strains that have developed resistance to several Cry proteins.[24]

Cry proteins bind to midgut cell surfaces within lepidopteran larvae, leading to cell death. Resistance typically arises through mutations in the receptor region within the midgut where Cry proteins bind. eCry1Gb.1Ig interacts with a different receptor than those targeted by Cry proteins currently expressed in GM crops, which explains its efficacy against resistant lepidopteran insects.[25]

A description of the inserted genetic elements is listed in the table below. The list is limited to the introduced genes and corresponding traits, and regulatory elements (promoters and terminators).

Introduced genes	**Encoded proteins**	**Regulatory elements**	**Traits**
eCry1Gb.1Ig-03 (eCry1Gb.1Ig)	codon-optimized chimera of Cry1Gb and Cry1Ig, sequences derived from *Bacillus thuringiensis*[24]	Ubiquitin promoter sequence (SoUbi4-02) from sugarcane (*Saccharum officinarum L.*)[26], ubiquitin terminator sequence (ZmUbi361-05) from maize (*Zea mays L.*)[27]	insecticidal activity against certain lepidopteran insects
pmi-15 (PMI)	phosphomannose isomerase (PMI) enzyme derived from *Escherichia coli* K 12[28]	ubiquitin promoter (Ubi1-43) and a ubiquitin terminator (Ubi1-04), both derived from maize (*Zea mays L.*)[29]	transformation selection marker: enables transformed plant cells to use mannose as a sole carbon source

3.4 Molecular characterisation

The applicant performed whole genome sequence (WGS) analyses of three generations of MZIR260. The sequencing results demonstrated a single integration site on chromosome 2 of the MZIR260 maize genome. No other unintended sequences such as plasmid backbone sequences were detected. The insert as well as the genome-to-insert junctions were identical for all three sequenced generations of MZIR260 maize, indicating stable inheritance of the insert over the generations.

Comparison of the MZIR260 insert (10,850 bp) and flanking regions (1,000 bp on both ends) final consensus sequence with the sequence of the transformation plasmid demonstrated that the insert in the MZIR260 maize genome is intact without any rearrangements. However, a single nucleotide substitution, guanine to thymine at position 1786 within the intron of the sugarcane-derived ubiquitin promoter, was found in the MZIR260 insert. Additionally, deletions were observed in sequences at both the right and left end of the pSYN24795 insert, located outside the expression cassettes of the MZIR260 insert.

To identify the location of the insert, the applicant used the Basic Local Alignment Search Tool for Nucleotides (BLASTN) and Translated Nucleotides (BLASTX) programs to screen 1,000 base pairs upstream and 1,000 base pairs downstream of the maize genomic sequences flanking the MZIR260 insert. The results indicate that the insert does not interrupt any known maize gene. Sequence analysis of the MZIR260 genomic insertion site demonstrated that a 30-base pair region from the native maize genomic sequence was deleted at the MZIR260 insertion site.

The applicant screened the 3' and 5' junctions of the insert and its flanking regions, as well as the entire insert, for potential newly created open reading frames (ORFs). According to the applicant, the putative products of the identified ORFs did not generate any protein sequence similarity with known allergens, toxins, or other biologically active proteins.

Overall, the molecular characterisation was conducted according to the criteria previously laid down by COGEM.[30] The results from the molecular characterisation do not provide indications that MZIR260 maize could pose a risk to the environment.

Conclusion: The molecular characterisation of maize MZIR260 is adequate and no indications for potential environmental risks were identified.

3.5 Phenotypic and agronomic characteristics

The applicant analysed the phenotypic and agronomic characteristics of maize MZIR260 through field trials. Germination rates and seed viability of MZIR260 were compared to those of non-GM reference maize varieties under different growing conditions, and were found to be comparable. The assessment concluded that there is not any biologically relevant difference in forage and grain composition between MZIR260 and conventional maize. The introduced traits do not provide indications that MZIR260 has an altered survivability compared to conventional maize, and do not allow maize MZIR260 to survive or establish in the Dutch environment.

Conclusion: The introduced traits in GM maize MZIR260 do not alter the survivability of the maize in the Netherlands.

4. Food/feed assessment

This application is submitted under Regulation (EC) 1829/2003, therefore a food/feed assessment is conducted 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 conducted by Wageningen Food Safety Research (WFSR). The outcome of the assessment by other organisations (EFSA, WFSR) was not known when this advice was completed.

5. Post-market environmental monitoring

The applicant supplied a general surveillance (GS) plan as part of the PMEM. COGEM has published several recommendations for further improvement of GS plans,[31,32] but considers the current GS and PMEM plan adequate for the import and processing of maize MZIR260.

6. Overall conclusion

Conclusion: COGEM is of the opinion that import and processing of maize MZIR260 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 conduct a food/feed assessment.

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