Environmental risk assessment of import and processing of insect resistant genetically modified maize MON 95275

COGEM advice CGM/221103-01

- The present application (EFSA/GMO/NL/2022/173) concerns the authorisation for import and processing for use in feed and food of genetically modified (GM) maize MON 95275;
- Maize MON 95275 will not be commercialised as a single product, the environmental risk assessment therefore considers the hypothetical case in which import could occur;

- Maize MON 95275 was produced by *Agrobacterium tumefaciens* mediated transformation;
- Maize MON 95275 expresses the *mpp75Aa1.1* and *vpb4Da2* genes and the *DvSnf7.1* suppression cassette, all of which confer resistance against feeding damage caused by certain coleopteran insects;

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

- The molecular characterisation of maize MON 95275 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 MON 95275 to survive in the Dutch environment;

- COGEM is of the opinion that import and processing of maize MON 95275 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.

1. Introduction

The present application (EFSA/GMO/NL/2022/173), filed by Bayer CropScience LP, as represented by Bayer Agriculture BV., concerns the import and processing of genetically modified (GM) maize MON 95275, for use in feed and food. The applicant notes that MON 95275 will not be commercialised as a single product, instead it will be used in stacked lines created by traditional breeding. In this application, hypothetical import is considered. Maize MON 95275 was produced by *Agrobacterium tumefaciens* mediated transformation, and expresses the *mpp75Aa1.1* and *vpb4Da2* genes, which confer resistance against feeding damage by certain coleopteran insects. MON 95275 also contains the *DvSnf7.1* suppression cassette, which expresses the double strand

RNA (dsRNA) transcript DvSnf7, which confers resistance against feeding damage caused by the Western corn rootworm (*Diabrotica virgifera virgifera*).

2. Previous COGEM advice

COGEM previously issued a positive advise on GM maize line MON 87411, which also expresses the dsRNA transcript DvSnf7,[1] and a stacked line containing MON 87411.[2] Regarding another stacked line containing MON 87411, COGEM was not able to perform an environmental risk assessment, because the bioinformatics analyses was incomplete.[3]

3. Environmental risk assessment

3.1 Characteristics of maize

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[4,5] 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.[6] Hybridisation of GM maize with other species than teosinte, the wild relative of maize, cannot occur.

Maize requires warm conditions in order to grow.[6,7] In cultivation areas with warm climatic conditions, the appearance of volunteers – not deliberately planted - 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 does not tolerate prolonged cold and frost.[8]

Maize is very sensitive to weed competition.[9] During the long process of domestication, maize has lost the ability to persist in the wild.[5] A soil seed bank, small seeds, and an extended period of flowering and seed production are characteristics often observed in persistent weeds.[10] Maize lacks all these characteristics. After ripening, the seeds (the kernels) adhere to the cob and do not scatter naturally.[6,11] Consequently, seed dispersal is severely hampered.

3.2 Receiving environment

In the Netherlands, the appearance of maize volunteers is rare, although maize plants occasionally have been observed outside agricultural fields.[12,13] Any volunteers emerging will be killed by frost at the onset of winter.[8] COGEM is not aware of any reports of feral maize populations in the Netherlands. Additionally, hybridisation of GM maize with teosinte is not possible, as teosinte is absent in maize fields and in nature in the Netherlands.[8]

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

3.3 Description of the introduced genes, traits and regulatory elements

GM maize MON 95275 was produced by *Agrobacterium tumefaciens* mediated transformation of immature maize embryo’s using the plasmid vector PV-ZMIR525664. This plasmid vector contains a single T-DNA, which comprises the expression cassettes *mpp75Aa1.1* and *vpb4Da2*, the *DvSnf7.1* suppression cassette, and the selectable marker *cp4 epsps* flanked by *loxP* sites. Selected plants containing the T-DNA were crossed with a maize line expressing Cre recombinase, which was developed using transformation plasmid PV-ZMOO513642. In the progeny, the selectable marker *cp4 epsps* and *cre* gene were excised. A description of the inserted genetic elements is listed in the table below. The list is limited to the introduced expression and suppression cassettes and corresponding traits, and regulatory elements (promoters and terminators).

Introduced casettes	**Encoded products**	**Traits**	**Regulatory elements**
DvSnf7b sequence	Expresses a dsRNA transcript that contains a fragment of the *Snf7* gene from *Diabrotica virgifera virgifera* encoding the Snf7 subunit of the ESCRT–III Complex.[14]	Resistance to certain coleopteran insects.	*35S* promoter from *Cauliflower mosaic virus* (CaMV) and E9 gene 3'UTR from *Pisum sativum.*
mpp75Aa1.1 gene	Encodes the insecticidal Mpp75Aa1.1 protein, originating from *Brevibacillus laterosporus.*[15] Member of the β-pore forming protein (PFP) family.	Resistance to certain coleopteran insects.	DaMv-1 enhancer from *Dahlia mosaic virus* (DaMV), and *RCc3-TdI* promoter from *Tripsacum dactyloides.* Also contains the *14-3-3c-Si1* intron from *Setaria italica*, and the *HSP-Cl1* 3' UTR from *Coix lacryma-jobi* for polyadenylation of the mRNA.
vpb4Da2 gene	Encodes the insecticidal Vpb4Da2 protein originating from *Bacillus thuringiensis.*[16] Member of the β-PFP family.	Resistance to certain coleopteran insects.	*DaMV-2* enhancer and *Ltp-Zm1* promoter from *Z. mays*, the *Act-Si1* intron from *S. italica*, and the *SAM1-Si1* 3' UTR from *S. italica* for polyadenylation of the mRNA.
See references for detailed descriptions of the traits.			

3.4 Molecular characterisation

The applicant characterised the DNA insert and flanking DNA in MON 95275 using Next Generation Sequencing (NGS), bioinformatic analyses, and directed sequencing using locus specific PCR. The molecular analyses showed that maize MON 95275 contains a single T-DNA insert, stably integrated at a single locus in chromosome 3 of the maize genome. With NGS, the applicant also demonstrated the absence of backbone sequences from the two plasmids used, including the selectable marker and *cre* sequences. Using directed sequencing, the applicant confirmed that the sequence of the T-DNA (14.682 bp) was almost identical to the corresponding region in the plasmid vector PV-ZMIR525664, except for a single nucleotide difference in a non-coding intervening sequence. Following a comparison of the insertion site with the genome of conventional maize, a 746 bp deletion of the maize genome was observed and an insertion of 6 bp within the 3’ flanking sequence, which occurred during transformation. Bioinformatic analysis of the flanking regions of the insert indicate that no endogenous genes are disrupted by the insertion of the T-DNA.

The applicant screened the junctions between the insert and the flanking regions as well as the entire insert for potential newly created open reading frames (ORFs), from stop codon to stop codon. These ORFs were evaluated for potential similarity to known toxins, allergens or biologically active proteins that could affect human or animal health. Two out of six ORFs of the T-DNA insert, encoding the insecticidal proteins Mpp75Aa1.1 and encoding Vpb4Da2, showed similarity to other related β-pore forming proteins in the toxin database. The applicant states that important differences were found in the receptor binding domains of the insecticidal proteins compared to the sequences retrieved from the toxin database, and that there are no indications that the insecticidal proteins could display toxicity towards mammals and other vertebrates. No other sequence similarities with known putative allergens, toxins or other biologically active proteins that may be harmful for human or animal health, were detected in these bioinformatic analyses.

Overall, the molecular characterisation was conducted according to the criteria previously laid down by COGEM.[17]

Conclusion: The molecular characterisation of maize MON 95275 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 MON 95275 through field trials. The introduced traits do not provide indications that MON 95275 has an altered survivability compared to conventional maize, and do not allow maize MON 95275 to survive or establish in the Dutch environment.

Conclusion: The introduced traits in GM maize MON 95275 do not alter survivability 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, a food and/or feed assessment for Regulation (EC) 1829/2003 applications is carried out 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 (PMEM)

The applicant supplied a general surveillance (GS) plan as part of the PMEM. COGEM has previously published several recommendations for further improvement of the GS plan,[18,19] but considers the current GS plan adequate for import and processing of maize MON 95275.

6. Overall conclusion

COGEM is of the opinion that import and processing of maize MON 95275 (a hypothetical case, as GM maize MON 95275 will not be commercialised as a standalone product) 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 regarding hypothetical import

COGEM notes that although an application for import and processing of MON 95275 was filed, the applicant states that it will not be commercialised as a single event and will only be used to create stacked events. This situation results from the procedures followed by EFSA, i.e. that an application for import and processing of a stacked GM line can only be filed if the parental GM lines have been assessed.[20] COGEM points out that in this particular case it is not relevant to assess the single event MON 95275, because it will never be commercialised as a standalone product. COGEM considers the request for authorisation of MON 95275 for import and processing and use in food and feed as an example of following unnecessary procedures.

References

1. COGEM (2015). Import and processing of genetically modified maize MON87411. COGEM advice CGM/151029-01
2. COGEM (2021). Environmental risk assessment of import and processing of genetically modified maize DP4114 x MON89034 x MON87411 x DAS-40278-9 and its subcombinations. COGEM advice CGM/210709-01
3. COGEM (2018). Import and processing of genetically modified maize MON87427 x MON89034 x 1507 x MON87411 x 59122 x DAS-40278-9 and sub-combinations. COGEM advice CGM/180806-03
4. Hin CJA (2001). Landbouwkundige risico's van uitkruising van GGO-gewassen. Centrum voor Landbouw en Milieu (CLM)
5. Treu R & Emberlin J (2000). Pollen dispersal in the crops Maize (*Zea mays*), Oil Seed Rape (*Brassica napus* ssp. *oleifera*), Potatoes (*Solanum tuberosum*), Sugar Beet (*Beta vulgaris* ssp. *vulgaris*) and Wheat (*Triticum aestivum*). Evidence from Publications. Soil Association
6. Andersson M *et al.* (2010). Gene flow between crops and their wild relatives. The John Hopkins University Press, Baltimore, Maryland, The United States of America
7. Miedema P (1982). The effect of low temperature on *Zea mays*. Adv. Agron. 35: 93-128
8. Huiting HF *et al.* (2018). Are teosinte and feral maize present in the Netherlands? COGEM report CGM 2018-06
9. CAB International (2007). Crop Protection Compendium. *Zea mays* (maize). CD-ROM edition, Wallingford
10. Kos SP *et al*. (2012). Can transgenic crops go wild? A literature study on using plant traits for weediness pre-screening. COGEM research report CGM 2012-01

11. Organisation for Economic Cooperation and Development (OECD) (2003). Consensus document on the biology of *Zea mays* ssp. *mays* (Maize)
12. Trtikova M *et al.* (2017). Teosinte in Europe - Searching for the origin of a novel weed. Sci. Rep. 7: 1560. doi: 10.1038/s41598-017-01478-w
13. van de Wiel CCM *et al*. (2011). Crop volunteers and climate change. Effects of future climate change on the occurrence of Maize, Sugar Beet and Potato volunteers in the Netherlands. COGEM research report 2011-11
14. Ramaseshadri P *et al*. (2013). Physiological and cellular responses caused by RNAi- mediated suppression of *Snf7* orthologue in western corn rootworm (*Diabrotica virgifera virgifera*) larvae. PLoS One. 8(1):e54270
15. Kouadio JL *et al.* (2021). Structural and functional characterization of Mpp75Aa1.1, a putative beta-pore forming protein from Brevibacillus laterosporus active against the western corn rootworm. PLoS ONE 16: e0258052
16. Yin Y *et al.* (2020). A new Bacillus thuringiensis protein for Western corn rootworm control. PLoS ONE 15: e0242791
17. COGEM (2014). Reconsideration of het molecular characterisation criteria for marketing authorisation of GM crops. COGEM topic report CGM/140929-02
18. COGEM (2010). General Surveillance. COGEM topic report CGM/100226-01
19. COGEM (2015). Advice on improving the general surveillance of GM crops. COGEM advice CGM/150601-02
20. European Food Safety Authority (EFSA) (2010). Scientific opinion. Guidance on the environmental risk assessment of genetically modified plants. The EFSA Journal 8: 1879