Import and processing of genetically modified maize DP202216xNK603xDAS-40278-9 and subcombinations

COGEM advice CGM/240415-01

- The present application (GMFF-2022-6232) concerns the authorisation of genetically modified (GM) maize DP202216xNK603xDAS-40278-9 and its subcombinations for import and processing for use in feed and food;
- The stacked event maize DP202216xNK603xDAS-40278-9 was produced by conventional crossbreeding of the three GM parental maize lines;
- Previously, COGEM advised positively on the import and processing of all three parental lines;

- The GM maize expresses a modified *pat* gene (*mo-pat*), the *aad-1* gene and two copies of the *cp4 epsps* gene, conferring tolerance to glyphosate, glufosinate-ammonium, 2,4 dichlorophenoxyacetic acid (2,4-D), and aryloxyphenoxypropionate (AOPP) containing herbicides. Furthermore, it expresses the *zmm28* gene leading to increased and extended production of the endogenous ZMM28 protein resulting in plants with an enhanced grain yield potential;

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

- The molecular characterisation of DP202216xNK603xDAS-40278-9 has been updated and meets the criteria of COGEM;
- The updated bioinformatic analysis did not provide indications of potential environmental risks;

- There are no indications that the introduced traits allow the stacked event maize DP202216xNK603xDAS-40278-9 to survive in the Netherlands;
- There are no indications that DP202216xNK603xDAS-40278-9 could establish feral populations in the Netherlands;

- COGEM is of the opinion that import and processing of maize DP202216xNK603xDAS-40278-9, and GM maize consisting of subcombinations of its parental GM maize lines 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 (GMFF-2022-6232) filed by Corteva Agriscience LCC concerns import and processing of DP202216xNK603xDAS-40278-9 (DP-2Ø2216-6xMON-ØØ6Ø3-6xDAS-4Ø278-9) maize and genetically modified (GM) maize consisting of its subcombinations. The GM maize was produced by conventional crossbreeding of the three genetically modified (GM) parental maize lines. It expresses the *mo-pat* and *aad-1* genes, and two copies of the *cp4 epsps* gene, conferring tolerance to glufosinate-ammonium, glyphosate, 2,4-D and AOPP containing herbicides. Furthermore, it expresses the *zmm28* gene leading to increased and extended production of the endogenous ZMM28 protein resulting in plants with an enhanced grain yield potential.[1,2]

The parental lines NK603[3] and DAS-40278-9[4], and the NK603xDAS-40278-9[5] subcombination have been authorised for import and processing for use in food and feed in the European Union. The import, food and feed of the parental line DP202216 is not authorized yet.

2. Previous COGEM advice

Previously COGEM advised positively on cultivation of the parental line NK603[6,7], and on import and processing of the parental lines DP202216[8] and DAS-40278-9[9]. COGEM also advised positively on the import and processing of several stacked events including MON89034x1507xNK603xDAS-40278-9[10] and MON89034x1507xMIR162x NK603xDAS-40278-9[11].

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,[12,13] 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.[14]

In Europe, no wild relatives of maize other than teosinte, are present and, therefore, hybridization of GM maize with other species cannot occur. Teosinte has been detected in Spain[15,16,17] and France.[18,19] In these countries, it is present as a weed in some arable fields. In the Netherlands, teosinte is not present in the environment.[20]

Maize requires warm conditions to grow.[14,21] In cultivation areas with warm 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.[14] Maize does not tolerate prolonged cold and frost.

Maize is very sensitive to weed competition.[22] During the long process of domestication, maize has lost the ability to persist in the wild.[13] A soil seed bank, small seeds, and an extended period of flowering and seed production are characteristics often observed in persistent weeds.[23] Maize lacks all these characteristics. After ripening, the seeds (the kernels) adhere to the cob and do not scatter naturally.[14,24] Consequently, seed dispersal is severely hampered.

During field observations in Austria some volunteers and maize plants were observed in non-agricultural habitats.[25] In the Netherlands, the appearance of volunteers is rare, although maize plants occasionally have been observed outside agricultural fields.[20,26] Any volunteers emerging will be killed by frost at the onset of winter.[20] COGEM is not aware of any reports of feral maize populations in the Netherlands.[20]

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

3.2 Description of the introduced genes, traits, and regulatory elements

Maize DP202216xNK603xDAS-40278-9 was created by conventional cross-breeding of the parental lines. For a description of the parental lines, see previous COGEM advices.[6,7,8,9,10,11] A description of the inserted genetic elements is listed in the table below. The list is limited to information on the introduced genes, corresponding traits, and regulatory elements (promotors and terminators).

Introduced genes	**Encoded proteins**	**Traits**	**Regulatory elements**
aad-1 (DAS-40278-9)	Aryloxyalkanoate dioxygenase (AAD-1) enzyme originating from *Sphingobium herbicidovorans*[27]	Cleavage of several synthetic auxins and certain 'aryloxyphenoxy-propionate' (AOPP) herbicides, resulting in a tolerance to several synthetic auxin acting herbicides like 2,4 dichlorophenoxyacetic acid' (2,4-D) and to AOPP containing herbicides	Ubiquitin promoter (ZmUbi1) and ZmPer5 3'UTR from *Zea mays*
cp4 epsps (NK603)	5-enolpyruvylshikimate-3-phospate synthase (EPSPS) from *Agrobacterium* sp. strain CP4[28,29]	Tolerance to glyphosate containing herbicides	Rice actin *(p-ract1)* promoter and intron from *Oryza sativa*, *ctp2* transit peptide from *(A. thaliana)* and nopaline synthase (*NOS* 3'UTR) terminator from *A. tumefaciens*
cp4 epsps L214P (NK603)	5-enolpyruvylshikimate-3-phospate synthase from *Agrobacterium* sp. strain CP4[28,29]	Tolerance to glyphosate containing herbicides	Enhanced *35S* (*e35S*) promoter from *Cauliflower mosaic virus* (CaMV),

			Zmhsp70 intron (*Zea mays*), *ctp2* transit peptide from *(A. thaliana)* nopaline synthase (*NOS* 3'UTR) terminator from *A. tumefaciens*
zmm28 (DP202216)	MADS-domain transcription factor gene originating from *Zea mays* encoding for ZMM28 protein[2,1,30,31]	Plants with enhanced grain yield potential	Constitutive promoter (*zm-gos2*) and intron from the ubiquitin gene 1 (*ubi*ZM1) from *Z. mays* and proteinase inhibitor II (*pin*II) gene terminator from *Solanum tuberosum*
mo-pat (DP202216)	Maize codon-optimised version of phosphinothricin acetyl transferase (*pat*) gene from *Streptomyces viridochromogenes*[32,33,34]	Tolerance to glufosinate-ammonium containing herbicides	Promoter, 5'UTR and intron from the *ubi*ZM1 gene from *Z. mays* and *pin*II terminator from *S. tuberosum*
See references for a detailed description of the traits			

3.3 Molecular characterisation

Previously, COGEM evaluated the molecular characterisation of each parental line and considered these to be adequate.[6,7,8,9,10,11]

The applicant sequenced the DP202216xNK603xDAS-40278-9 maize and compared the obtained DP202216, NK603 and DAS-40278-9 inserts and flanking sequences in DP202216xNK603xDAS-40278-9 with the sequences of the corresponding single events. According to the applicant the DP202216, NK603 and DAS-40278-9 inserts and flanking DNA regions in the stacked event maize are identical to the DNA sequences determined previously for the respective single events.

The applicant updated the bioinformatics analyses of the DP202216, NK603 and DAS-40278-9 inserts and of the 5' and 3' flanking regions using recent databases. According to the applicant, the putative products of the open reading frames spanning the 5' and 3' junctions of the inserts did not generate any protein sequence similarity with known allergens, toxins, or other biologically active proteins.

The molecular characterisation was conducted according to the criteria previously laid down by COGEM.[35] The results from the updated molecular characterisation do not provide indications that the stacked event maize DP202216xNK603xDAS-40278-9 could pose a risk to the environment.

Conclusion: The molecular characterisation of maize DP202216xNK603xDAS-40278-9 is adequate and no indications off potential environmental risks were identified.

3.4 Phenotypic and agronomic characteristics

Previously, COGEM evaluated the phenotypic and agronomic characteristics of each parental line of DP202216xNK603xDAS-40278-9, and found no deviations influencing the outcome of the environmental risk assessment.

The applicant analysed the phenotypic and agronomic characteristics of DP202216xNK603xDAS-40278-9 and noted that these, except for the introduced traits, are not different to the non-GM near-isogenic control line, and are equivalent to the reference varieties, taking into account natural variation. The results of the phenotypic evaluation do not give reason to assume that the GM maize could pose an environmental risk.

Therefore, COGEM is of the opinion that there are no indications that the introduced traits allow maize DP202216xNK603xDAS-40278-9 to survive or establish in the Dutch environment.

Conclusion: There are no indications that the introduced traits allow DP202216xNK603xDAS-40278-9 to survive in the Netherlands. DP202216xNK603xDAS-40278-9 has no increased potential for the establishment of feral populations 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 plan as part of the PMEM. COGEM has published several recommendations for further improvement of the general surveillance (GS) plan,[36,37] but considers the current GS plan adequate for the import and processing of maize DP202216xNK603xDAS-40278-9.

6. Overall conclusion

COGEM is of the opinion that import and processing of DP202216xNK603xDAS-40278-9 maize and GM maize consisting of subcombinations of its parental GM maize lines pose 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.

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