Advice Import and processing of GM maize Bt11 x MIR162 x MON89034 x NK603 and its subcombinations

COGEM advice CGM/251105-02

COGEM has been requested to evaluate the environmental risks associated with the authorisation for import, processing and food and feed use of genetically modified (GM) maize Bt11 x MIR162 x MON89034 x NK603 and its subcombinations, GMFF-2025-33624 (MA 250015), as submitted by Syngenta Crop Protection NV/SA on behalf of Syngenta Crop Protection AG.

GM maize Bt11 x MIR162 x MON89034 x NK603 was produced by crossing the four GM parental maize lines using conventional breeding methods. The GM maize expresses the *cry1Ab* gene derived from Bt11, the gene *vip3Aa20* derived from MIR162 and the transgenes *cry1A.105* and *cry2Ab2* derived from MON 89034 which confer resistance against certain lepidopteran pests (Table 1). The GM maize also expresses *pat* gene derived from Bt11 and the *cp4 epsps* and *cp4 esps L214P* genes derived from NK603 which confer tolerance to glufosinate-ammonium containing herbicides and glyphosate containing herbicides, respectively. The *cp4 epsps* and *cp4 esps L214P* genes differ one amino acid on position 214 and are functionally equivalent. In addition, GM maize Bt11 x MIR162 x MON89034 x NK603 contains the *pmi* derived from MIR162 which was used as a selectable marker during development of MIR162.

Table 1. Description of the introduced genes and traits

Introduced genes	Encoded proteins	Regulatory elements	Traits
cry1Ab (Bt11)	A variant of the Cry1Ab protein from *Bacillus thuringiensis* subsp. *kurstaki*[1]	*35S* promoter from cauliflower mosaic virus (CaMV) and nopaline synthase (*nos*) terminator from *Agrobacterium tumefaciens*	Resistance to certain lepidopteran insects
pat (Bt11)	Variant of phosphinothricin acetyl transferase (PAT) originating from *Streptomyces viridochromogenes* strain Tü 494[2,3,4,5]	*35S* promoter from CaMV and *nos* terminator from *A. tumefaciens*	Tolerance to glufosinate-ammonium containing herbicides
cry1A.105 (MON89034)	The Cry1A.105 protein is a chimeric protein with domains from different Cry1 proteins from *B. thuringiensis* subsp. *kumamotoensis*[6]	Enhanced *35S* (*e35S*) promoter from CaMV and T-Hsp17 terminator from *Triticum aestivum*	Resistance to certain lepidopteran insects

Introduced genes	**Encoded proteins**	**Regulatory elements**	**Traits**
cry2Ab2 (MON89034)	Variant of the Cry2Ab2 protein from *B. thuringiensis* subsp. *kurstaki*[6]	35S promoter from *Figwort mosaic virus* (FMV) and *nos* terminator from *A. tumefaciens*	Resistance to certain lepidopteran insects
vip3Aa20 (MIR162)	Variant of a native vegetative insecticidal protein (Vip) class A, subclass a, (Vip3Aa20) originating from *B. thuringiensis* strain AB88[7]	Polyubiquitin promoter and intron (Zm*Ubi*Int) from *Zea mays* and *35S* terminator from CaMV	Resistance to certain lepidopteran insects
pmi, also known as *manA* (MIR162)	Phosphomannose isomerase (PMI) enzyme derived from *Escherichia coli* K12[8]	Polyubiquitin promoter and intron (Zm*Ubi*Int) from *Z. mays* and nopaline synthase (*nos*) terminator from *A. tumefaciens*	Selectable marker: enables transformed plant cells to use mannose as a sole carbon source
cp4 epsps (NK603)	The 5-enolpyruvulshikimate-3-phosphate synthase (EPSPS) enzyme originating from *A. tumefaciens* strain CP4[9,10]	*Ract1* promoter from *Oryza sativa* and *nos* terminator from *A. tumefaciens*	Tolerance to glyphosate containing herbicides
cp4 epsps L214P (NK603)	The 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) L214P enzyme originating from *A. tumefaciens* strain CP4[9,10], functionally equivalent to the EPSPS enzyme.	*E35S* promoter from CaMV and *nos* terminator from *A. tumefaciens*	Tolerance to glyphosate containing herbicides

COGEM has previously advised positively on the cultivation and import of NK603[11,12,13] and MON89034[14,15], and on import and processing of the parental lines Bt11,[16,17,18,19] and MIR162.[20,21] COGEM also advised positively on the import and processing of many stacked events containing these parental lines.[e.g.22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54] The parental lines NK603, MON89034, Bt11 and MIR162 have all been authorised for import and processing for use in food and feed in the European Union.[55,56,57,58]

Maize (*Zea mays*) is a highly domesticated crop that is cultivated globally. Maize is wind pollinated.[59,60] Insect pollination of maize is highly limited but cannot be excluded.[61] Maize is very sensitive to weed competition.[62] During the long process of domestication, maize has lost the ability to persist in the

wild.[60] After ripening, the seeds (the kernels) adhere to the cob and do not scatter naturally.[61,63] Consequently, seed dispersal is severely hampered.
Maize does not tolerate prolonged cold and frost, and requires warm conditions to grow.[61,64,65] 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.[61] In the Netherlands the appearance of volunteers is rare, although maize plants occasionally have been observed outside agricultural fields.[67,66] Any volunteers emerging will be killed by frost at the onset of winter.[67] COGEM is not aware of any reports of feral maize populations in the Netherlands.[67] Hybridisation of GM maize with species other than teosinte – the wild relative of maize – cannot occur. However, as teosinte is absent in maize fields and nature in the Netherlands,[67] hybridisation of GM maize with teosinte will not occur in the Netherlands.

The bio-informatic analysis of each of the inserted elements and its 3' and 5' junctions in GM maize Bt11 x MIR162 x MON89034 x NK603 was updated, using up-to-date databases of allergens, toxins, and other biologically active proteins to assess protein sequence similarities. No indications for potential environmental risks were identified.
As a minor comment on the bio-informatics analysis, COGEM notes that the applicant reports that, for the allergenicity assessment, a low complexity alignment was found between MIR162 and the sequences of two allergens. The applicant does not consider low complexity alignments to be indicative of shared structure or function, and therefore regards such protein sequence similarities as biologically irrelevant. However, COGEM is of the opinion that the biological relevance of low complexity alignments has been insufficiently addressed by the applicant, as there is no scientific consensus on the significance of low complexity regions.

The applicant analysed the phenotypic and agronomic characteristics of Bt11 x MIR162 x MON89034 x NK603 and reported that most characteristics of the GM maize did not differ from the non-transgenic, near-isogenic control maize (conventional counterpart), and is equivalent to the reference varieties, taking natural variation into account. An exception is reported for 'final stand count' (number of plants per m^2), for which equivalence could not be established. The observed difference of 0.21 plants/m^2 is less than one plant/m^2 and is not considered to have any agronomical or biological relevance.

There are no indications that the introduced traits in maize Bt11 x MIR162 x MON89034 x NK603 will allow the GM maize to survive in the Dutch environment. A post-market environmental monitoring (PMEM) plan is provided in the application.

COGEM is of the opinion that renewal of the market authorisation for import and processing of GM maize Bt11 x MIR162 x MON89034 x NK603 and GM maize consisting of subcombinations of its parental GM maize lines pose a negligible risk to the Dutch environment. COGEM abstains from giving advice on the potential risks of incidental consumption, as a food/feed assessment is carried out by other organisations.

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