

Advice Environmental risk assessment of import and processing of modified maize Bt11 x MIR162 x 1507 x NK603 and its subcombinations

COGEM advice CGM/250515-03

- The present application (GMFF-2024-22091) concerns the authorisation of genetically modified (GM) maize Bt11 x MIR162 x 1507 x NK603 and its subcombinations for import and processing for use in feed and food;
- The stacked event maize Bt11 x MIR162 x 1507 x NK603 was produced by conventional crossbreeding of the four GM parental maize lines;
- Previously, COGEM has advised positively on the import and processing of all four parental lines.
- The GM maize expresses the *cry1Ab*, *cry1F* and *vip3Aa20* genes, which each code for proteins conferring resistance against certain lepidopteran pests. Furthermore, the GM maize expresses the *cp4 epsps* gene and two copies of the *pat* gene, which encode proteins conferring tolerance to glyphosate and glufosinate-ammonium containing herbicides, respectively. Lastly, it expresses the *pmi* gene, which encodes a protein that enables cells to utilise mannose as a primary carbon source. This trait was used as a selectable marker in the development of the maize line.
- In the Netherlands, feral maize populations have never been observed and the appearance of volunteer maize plants is rare. Additionally, the wild relative of maize, teosinte is not present in the Dutch environment. Hybridisation of GM maize with other species is therefore not possible.
- The molecular characterisation of Bt11 x MIR162 x 1507 x NK603 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 Bt11 x MIR162 x 1507 x NK603 to survive in the Netherlands;
- There are no indications that Bt11 x MIR162 x 1507 x NK603 could establish feral populations in the Netherlands.
- COGEM is of the opinion that import and processing of maize Bt11 x MIR162 x 1507 x NK603, as well as 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, as a food/feed assessment is carried out by other organisations.

1. Introduction

The present application (GMFF-2024-22091), filed by Syngenta, concerns the import and processing of genetically modified (GM) maize Bt11 x MIR162 x 1507 x NK603 (SYN-BTØ11-1 x SYN-IR162-4 x DAS-Ø15Ø7-1 x MON-ØØ6Ø3-6) and its subcombinations. The GM maize was produced by conventional crossbreeding of the four GM parental maize lines. It contains the *cry1Ab*, *cry1F*, and *vip3Aa20* genes, each coding for proteins conferring resistance against certain lepidopteran pests. It also contains the *cp4 epsps* gene and two copies of the *pat* gene, which code for proteins conferring tolerance to glyphosate and glufosinate-ammonium containing herbicides, respectively. Lastly, the maize contains the *pmi* gene which encodes a protein that enables cells to utilize mannose as a primary carbon source, which was used as a selectable marker in the development of the line.

The parental lines NK603, 1507, Bt11, MIR162, the double stacked events NK603 x 1507, NK603 x Bt11, NK603 x MIR162, Bt11 x 1507, Bt11 x MIR162, 1507 x MIR162, and the triple stacked events NK603 x 1507 x MIR162, 1507 x MIR162 x Bt11, and all their possible subcombinations have been authorised for import and processing for use in food and feed in the European Union.

2. Previous COGEM advice

Previously COGEM advised positively on the cultivation and import of NK603^{1,2,3} and 1507^{4,5,6} and on import and processing of the parental lines Bt11,^{7,8,9,10} and MIR162.^{11,12} COGEM also advised positively on the import and processing of many stacked events containing these parental lines.^{13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46}

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 from Central America, and is nowadays cultivated globally. Maize is wind pollinated,^{47,48} 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.⁴⁹ Hybridisation of GM maize with species other than teosinte – the wild relative of maize – cannot occur.

Maize does not tolerate prolonged cold and frost, and requires warm conditions to grow.^{50,49,51} In cultivation areas with warm climatic conditions, volunteers, i.e. 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.⁴⁹

Maize is very sensitive to weed competition.⁵² During the long process of domestication, maize has lost the ability to persist in the wild.⁴⁸ A soil seed bank, small seeds, and an extended period of flowering and seed production are characteristics often observed in persistent weeds.⁵³ Maize lacks all these characteristics. After ripening, the seeds (the kernels) adhere to the cob and do not scatter naturally.^{49,54} Consequently, seed dispersal is severely hampered.

3.2 Receiving environment

In the Netherlands, the appearance of volunteers is rare, although maize plants occasionally have been observed outside agricultural fields.^{56,55} Any volunteers emerging will be killed by frost at the onset of winter.⁵⁶ COGEM is not aware of any reports of feral maize populations in the Netherlands.⁵⁶

Maize can hybridise with teosinte, the wild relative of maize. However, as teosinte is absent in maize fields and nature in the Netherlands,⁵⁶ hybridisation of GM maize with teosinte will not occur in the Netherlands.

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

3.3 Description of the introduced genes and traits

GM maize Bt11 x MIR162 x 1507 x NK603 was created by conventional crossbreeding of the parental lines. For a description of the development of the parental lines, see previous COGEM advices (referenced in paragraph 2). A description of the inserted genetic elements in GM maize Bt11 x MIR162 x 1507 x NK603 is listed in the table below. The list is limited to information on the introduced genes, corresponding traits, and regulatory elements (promoters and terminators).

Introduced genes	Encoded proteins	Regulatory elements	Traits
<i>cry1Ab</i> (Bt11)	A variant of the Cry1Ab protein from <i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> ⁵⁷	35S promoter from Cauliflower mosaic virus (CaMV) and nopaline synthase (<i>nos</i>) terminator from <i>Agrobacterium tumefaciens</i>	Resistance to certain lepidopteran insects
<i>pat</i> (Bt11)	Variant of phosphinothricin acetyl transferase (PAT) originating from <i>Streptomyces viridochromogenes</i> strain Tü 494 ^{58,59,60}	35S promoter from Cauliflower mosaic virus (CaMV) and <i>nos</i> terminator from <i>A. tumefaciens</i>	Tolerance to glufosinate-ammonium containing herbicides

<i>vip3Aa20</i> (MIR162)	Variant of a native vegetative insecticidal protein (Vip) class A, subclass a, (Vip3Aa20) originating from <i>Bacillus thuringiensis</i> strain AB88 ⁶¹	Polyubiquitin promoter and intron (ZmUbiInt) from <i>Zea mays</i> and 35S terminator from <i>Cauliflower mosaic virus</i> (CaMV)	Resistance against certain lepidopteran insects
<i>pmi</i> , also known as <i>manA</i> (MIR162)	Phosphomannose isomerase (PMI) enzyme derived from <i>Escherichia coli</i> K12 ⁶²	Polyubiquitin promoter and intron (ZmUbiInt) from <i>Z. mays</i> and nopaline synthase (<i>nos</i>) terminator from <i>A. tumefaciens</i>	Enables transformed plant cells to use mannose as a sole carbon source
<i>cp4 epsps</i> (NK603)	The 5-enolpyruvulshikimate-3-phosphate synthase (EPSPS) enzyme originating from <i>A. tumefaciens</i> strain CP4 ^{63,64}	<i>Ract1</i> promoter from <i>Oryza sativa</i> and <i>nos</i> terminator from <i>A. tumefaciens</i>	Tolerance to glyphosate containing herbicides
<i>cp4 epsps L214P</i> (NK603)	The 5-enolpyruvulshikimate-3-phosphate synthase (EPSPS) L214P enzyme originating from <i>A. tumefaciens</i> strain CP4 ^{63,64} , functionally equivalent to the EPSPS enzyme.	E35S promoter from CaMV and <i>nos</i> terminator from <i>A. tumefaciens</i>	Tolerance to glyphosate containing herbicides
<i>cry1F</i> (1507)	A variant of the Cry1F protein originating from <i>B. thuringiensis</i> subsp. <i>aizawa</i> ⁶⁵	Polyubiquitin (<i>ubiZM1</i>) promoter and intron from <i>Z. mays</i> and ORF25 terminator from <i>A. tumefaciens</i> strain pTi15955	Resistance to certain lepidopteran insects
<i>pat</i> (1507)	Variant of phosphinothricin N-acetyltransferase (PAT) originating from <i>Streptomyces viridochromogenes</i> strain Tü 494 ^{58,59,60}	35S promoter and 35S terminator from CaMV	Tolerance to glufosinate-ammonium containing herbicides

3.4 Molecular characterisation

COGEM has previously evaluated the molecular characterisation of each parental line and considered them to be adequate.^{3,6,10,12}

The applicant updated the bioinformatics analyses of the Bt11, NK603, 1507, and MIR162 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.⁶⁶ The results from the updated molecular characterisation do not provide indications that the stacked event maize Bt11 x MIR162 x 1507 x NK603 could pose a risk to the environment.

Conclusion: The molecular characterisation of GM maize Bt11 x MIR162 x 1507 x NK603 is adequate and no indications of potential environmental risks were identified.

3.5 Phenotypic and agronomic characteristics

Previously, COGEM evaluated the phenotypic and agronomic characteristics of each parental line of Bt11 x MIR162 x 1507 x NK603 and found no deviations influencing the outcome of the environmental risk assessment.

The applicant analysed the phenotypic and agronomic characteristics of Bt11 x MIR162 x 1507 x NK603 and noted that these, except for the introduced traits, are not different compared to the non-GM near-isogenic control line, and are equivalent to the reference varieties, taking natural variation into account. 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 Bt11 x MIR162 x 1507 x NK603 to survive or establish in the Dutch environment.

Conclusion: There are no indications that the introduced traits allow Bt11 x MIR162 x 1507 x NK603 to survive in the Netherlands. Bt11 x MIR162 x 1507 x NK603 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 the European Food and Safety Authority (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 published several recommendations for further improvement of the GS plan,^{67,68} but considers the current GS and PMEM plan adequate for the import and processing of GM maize Bt11 x MIR162 x 1507 x NK603.

Conclusion: The current PMEM plan is sufficient for the import and processing of GM maize Bt11 x MIR162 x 1507 x NK603.

6. Overall conclusion

Conclusion: COGEM is of the opinion that import and processing of Bt11 x MIR162 x 1507 x NK603 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.

References

1. COGEM (2003). Markttoelating 'NK603 maize tolerant to glyphosate'. [In Dutch] COGEM advice CGM/030319-08
2. COGEM (2006). Cultivation of herbicide tolerant maize line NK603. COGEM advice CGM/060704-01
3. COGEM (2024). Renewal of the authorisation for import and processing of genetically modified maize NK603. COGEM advice CGM/240812-01
4. COGEM (2003). Insect resistant and glufosinate ammonium tolerant transformation event 1507 maize. [In Dutch] COGEM advice CGM/030115-01
5. COGEM (2016). Application for cultivation of genetically modified maize 1507: assessment of COGEM advice in view of EFSA opinions. COGEM advice CGM/160929-03
6. COGEM (2015). Renewal of the authorisation for import and processing of genetically modified maize line 1507. COGEM advice CGM/150928-01
7. COGEM (1997). Advies C/GB/96/M4-01 betreffende het in het handelsverkeer brengen van genetisch gemodificeerde maïs waarin het cry-IA(b) gen (Bt-toxine) en het pat gen tot expressie komen [In Dutch]. COGEM advice CGM/970204-06
8. COGEM (2008). Renewal of authorization for import and processing of maize Bt11. COGEM advice CGM/080523-02
9. COGEM (2009). Renewal of authorization for import and processing of maize Bt11: additional information. COGEM advice CGM/090310-01
10. COGEM (2019). Second renewal of the authorisation for import and processing of genetically modified maize Bt11. COGEM advice CGM/190211-01
11. COGEM (2010). Import and processing of genetically modified maize MIR162. COGEM advice CGM/101019-04
12. COGEM (2021). Renewal of the authorisation for import and processing of genetically modified maize MIR162. COGEM advice CGM/210927-01
13. COGEM (2005). Import and processing of insect resistant and herbicide tolerant maize MON863 x MON810 x NK603. COGEM advice CGM/050228-02
14. COGEM (2005). Import and processing of insect resistant and herbicide tolerant maize MON863 x NK603. COGEM advice CGM/050228-03
15. COGEM (2007). Advice cultivation of maize variety 59122xNK603x1507. COGEM advice CGM/070925-03
16. COGEM (2007). Cultivation of genetically modified maize 1507 x 59122. COGEM advice CGM/071119-02
17. COGEM (2007). Import and processing of MON89034xNK603. COGEM advice CGM/071022-03
18. COGEM (2007). Import of genetically modified maize 59122 x 1507 x NK603. COGEM advice CGM/070817-01
19. COGEM (2007). Import of genetically modified maize 59122 x 1507. COGEM advice CGM/070911-02
20. COGEM (2007). Import of genetically modified maize 59122 x NK603. COGEM advice CGM/070817-02
21. COGEM (2008). Cultivation of genetically modified maize line 1507x59122. COGEM advice CGM/080325-02
22. COGEM (2008). Import and processing of maize Bt11xGA21. COGEM advice CGM/080417-01
23. COGEM (2009). Additional advice on the import and processing of MON89034xNK603. COGEM advice CGM/091020-01
24. COGEM (2009). Cultivation of maize line MON89034xNK603. COGEM advice CGM/091208-01
25. COGEM (2009). Import and processing of genetically modified maize Bt11xMIR162xGA21. COGEM advice CGM/090917-04
26. COGEM (2011). Additional information concerning the application for cultivation of MON89034xNK603 maize. COGEM advice CGM/110803-01
27. COGEM (2012). Additional information concerning the application for cultivation of genetically modified MON89034xNK603 maize. COGEM advice CGM/120327-01
28. COGEM (2012). Import and processing of genetically modified maize line 1507 x 59122 x MON810 x NK603. COGEM advice CGM/120402-01
29. COGEM (2012). Import and processing of genetically modified maize line Bt11x59122xMIR604x1507xGA21. COGEM advice CGM/120816-02
30. COGEM (2012). Import and processing of genetically modified maize line Bt11xMIR162x1507xGA21. COGEM advice CGM/120816-01
31. COGEM (2015). Import and processing of insect resistant and herbicide tolerant maize MON87427xMON89034xNK603. COGEM advice CGM/150817-01
32. COGEM (2016). Import and processing of genetically modified maize MON87427xMON89034xMIR162xNK603. COGEM advice CGM/160824-01

33. COGEM (2016). Import and processing of insect resistant and herbicide tolerant generically modified maize 1507xMON810xMIR162xNK603. COGEM advice CGM/160412-01
34. COGEM (2017). Import and processing of genetically modified maize MON89034x1507xMON88017x59122xDAS-40278-9. COGEM advice CGM/170814-01
35. COGEM (2017). Import and processing of genetically modified maize MON89034x1507xNK603xDAS-40278-9. COGEM advice CGM/170131-01
36. COGEM (2017). Renewal of the authorisation for import of feed of genetically modified 1507xNK603 maize. COGEM advice CGM/170731-02
37. COGEM (2017). Renewal of the authorisation for import of feed of genetically modified NK603xMON810 maize. COGEM advice CGM/170628-01
38. COGEM (2018). Import and processing of genetically modified maize Bt11xMIR162xMIR604x1507x5307x GA21. COGEM advice CGM/180430-02
39. COGEM (2018). Import and processing of genetically modified maize Bt11xMIR162xMIR604xMON89034x 5307xGA21 and sub-combinations. COGEM advice CGM/180924-01
40. COGEM (2018). Import and processing of genetically modified maize DP4114xMON810xMIR604xNK603. COGEM advice CGM/181019-01
41. COGEM (2018). Import and processing of genetically modified maize MON87427xMON89034x1507xMON87411x59122xDAS-40278-9 and sub-combinations. COGEM advice CGM/180806-03
42. COGEM (2018). Import and processing of genetically modified maize MON89034x1507xMIR162xNK603x DAS-40278-9 and sub-combinations. COGEM advice CGM/181220-03
43. COGEM (2023). Renewal of the authorisation for import and processing of genetically modified maize MON89034x1507xMON88017x59122 and part of its subcombinations. COGEM advice CGM/230622-01
44. COGEM (2023). Renewal of the authorisation for import and processing of genetically modified maize MON89034x1507xNK603. COGEM advice CGM/230711-01
45. COGEM (2024). Import and processing of genetically modified maize DP202216xNK603xDAS-40278-9 and subcombinations. COGEM advice CGM/240415-01
46. COGEM (2024). Renewal of the authorisation for import and processing of genetically modified maize NK603xT25. COGEM advice CGM/241011-01
47. Hin CJA (2001). Landbouwkundige risico's van uitkruising van GGO-gewassen. [In Dutch] Centrum voor Landbouw en Milieu (CLM)
48. 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
49. Andersson M & Carmen de Vicente M (2010). Gene flow between crops and their wild relatives. The John Hopkins University Press, Baltimore, Maryland, The United States of America
50. Huiting HF et al. (2018). Are teosinte and feral maize present in the Netherlands? COGEM report CGM 2018-06
51. Miedema P (1982). The effect of low temperature on *Zea mays*. Advances in Agronomy 35: 93-128
52. CAB International (2007). Crop Protection Compendium. *Zea mays* (maize). CD-ROM edition, Wallingford
53. 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
54. Organisation for Economic Cooperation and Development (OECD) (2003). Consensus document on the biology of *Zea mays* ssp. *mays* (Maize)
55. 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 report 2011-11
56. Huiting HF et al. (2018). Are teosinte and feral maize present in the Netherlands? COGEM report CGM 2018-06
57. Perlak FJ et al. (1991). Modification of the coding sequence enhances plant expression of insect control protein genes. Proc. Natl. Acad. Sci. U.S.A. 88: 3324-3328
58. Wohlleben W et al. (1988). Nucleotide sequence of the phosphinothricin N-acetyltransferase gene form *Streptomyces viridochromogenes* Tü494 and its expression in *Nicotiana tabacum*. Gene 70: 25-37
59. Eckes P et al. (1989). A synthetic gene confers resistance to the broad spectrum herbicide L-phosphinothricin in plants. J. Cell. Biochem. Supplement 13D, 334, Abstract M516
60. Organisation for Economic Cooperation and Development (OECD) (1999). Consensus document on general information concerning the genes and their enzymes that confer tolerance to phosphinothricin herbicide.

61. Estruch JJ *et al.* (1996). Vip3A, a novel *Bacillus thuringiensis* vegetative insecticidal protein with a wide spectrum of activities against lepidopteran insects. *Proc. Natl. Acad. Sci. U.S.A.* 93: 5389-5394
62. Negrotto M *et al.* (2000). The use of phosphomannose-isomerase as a selectable marker to recover transgenic maize plants (*Zea mays* L.) via *Agrobacterium* transformation. *Plant Cell Reports* 19: 798-803
63. Funke T *et al.* (2006). Molecular basis for the herbicide resistance of Roundup Ready crops. *Proc. Natl. Acad. Sci. U.S.A.* 103: 13010-13015
64. Harrison LA, Bailey MR, Leimgruber RM, Smith CE, Nida DL, Taylor ML, Gustafson M, Heeren B and Padgett SR, 1993. Characterization of Microbially-Expressed Protein: CP4 EPSPS. Monsanto Company, MSLo012901.
65. US-EPA (U.S. Environmental Protection Agency). Biopesticide registration action document, Cry1Ab and Cry1F *Bacillus thuringiensis* (Bt) corn plant-incorporated protectants (2010). https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/decision_PC-006481_1-Sep-10.pdf (visited: March 13th, 2025)
66. COGEM (2014). Reconsideration of the molecular characterisation criteria for marketing authorisation of GM crops. COGEM report CGM/140929-02
67. COGEM (2010). General Surveillance. COGEM report CGM/100226-01
68. COGEM (2015). Advice on improving the general surveillance of GM crops. COGEM advice CGM/150601-02