COMMISSIE
COGEM
GENETISCHE
MODIFICATIE

BEZOEKADRES:
A. VAN LEEUWENHOEKLAAN 9
3721 MA BILTHOVEN

POSTADRES:
POSTBUS 578
3720 AN BILTHOVEN

TEL.: 088 689 2777
INFO@COGEM.NET
WWW.COGEM.NET

Aan de staatssecretaris van
Infrastructuur en Waterstaat
drs. V.L.W.A. Heijnen
Postbus 20901
2500 EX Den Haag

DATUM	13 juli 2023
KENMERK	CGM/230713-01
ONDERWERP	Advies import en verwerking van gg-mais MON94804

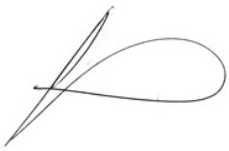
Geachte mevrouw Heijnen,

Naar aanleiding van een vergunningaanvraag voor import en verwerking van genetisch gemodificeerde maïs MON94804 (GMFF-2022-10651), ingediend door Bayer Agriculture BV, namens Bayer CropScience LP, deelt de COGEM u het volgende mee.

Samenvatting:
De COGEM is gevraagd om te adviseren over eventuele milieurisico's van import en verwerking van genetisch gemodificeerde (gg-) maïs MON94804. MON94804 planten blijven kleiner dan conventionele maïsplanten, doordat MON94804 een *GA20ox_SUP* suppressiecassette bevat die de productie van het plantenhormoon gibberelline onderdrukt. MON94804 zal worden gebruikt om kruisingslijnen te maken en wordt niet als individuele lijn gecommercialiseerd.
De moleculaire karakterisering van maïs MON94804 voldoet aan de eisen van de COGEM. Verwildering van maïsplanten is in Nederland nooit waargenomen. Er zijn geen redenen om aan te nemen dat expressie van de ingebrachte suppressiecassette ervoor zorgt dat gg-maïs MON94804 zich in Nederland zou kunnen vestigen. De wilde verwant van maïs, teosinte, komt niet in de natuurlijke omgeving in Nederland voor, waardoor de kans dat de ingebrachte genetische eigenschap zich naar een andere soort kan verspreiden, te verwaarlozen is.
Gezien het bovenstaande, acht de COGEM de milieurisico's bij import en verwerking van gg-maïs MON94804 verwaarloosbaar klein. Omdat een voedselveiligheidsbeoordeling door andere instanties wordt uitgevoerd, heeft de COGEM bij deze vergunningaanvraag de risico's van incidentele consumptie niet beoordeeld.

De door de COGEM gehanteerde overwegingen en het hieruit voortvloeiende advies treft u hierbij aan als bijlage.

Hoogachtend,



Prof. dr. ing. Sybe Schaap
Voorzitter COGEM

c.c.

- Drs. Y de Keulenaar, Hoofd Bureau ggo
- Ministerie van IenW, Directie Omgevingsveiligheid en milieurisico's, DG Milieu en Internationaal
- Ing. M.A.C. Möllers, Food-Feed loket

Environmental risk assessment of import and processing of GM maize MON94804

COGEM advice CGM/230713-01

- The present application (GMFF-2022-10651) concerns the authorisation for import and processing for use in food and feed of genetically modified (GM) maize MON94804;
- MON94804 will be used as a parental line to generate stacked events, but will not be commercialised as a single product;

- Maize MON94804 was produced by *Agrobacterium*-mediated transformation, and expresses a *GA20ox_SUP* suppression cassette, which down-regulates the expression of the *ZmGA20ox3* and *ZmGA20ox5* genes and results in reduced levels of the plant hormone gibberellic acid/gibberellin. As a consequence, MON94804 has a reduced plant height;

- 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, hybridisation of GM maize with other species is therefore not possible;

- The molecular characterisation of maize MON94804 meets the criteria of COGEM, and no indications for potential environmental risks were identified;
- There are no indications that the introduced trait will allow GM maize MON94804 to survive in the Dutch environment;

- COGEM is of the opinion that import and processing of maize MON94804 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 (GMFF-2022-10651), filed by Bayer Agriculture BV on behalf of Bayer CropScience LP, concerns the import and processing of genetically modified (GM) maize MON94804, for use in food and feed. The applicant notes that this event will be used as a parental line to generate stacked events but will not be commercialised as a single product.

MON94804 was produced by *Agrobacterium tumefaciens* mediated transformation, and contains a *GA20ox_SUP* suppression cassette, which down-regulates the expression of the *ZmGA20ox3* and *ZmGA20ox5* genes. This leads to reduced levels of gibberellic acid/gibberellin resulting in a reduction of internode length and reduced overall plant height.

2. Previous COGEM advice

COGEM did not previously assess GM events with reduced gibberellic acid/gibberellin levels or other traits resulting in a reduced plant height. COGEM did assess several GM events with suppression cassettes directed at other traits. Positive opinions were published on GM soybean MON87705 which has an altered fatty acid profile,[1] GM potato BPS-A1020-5 which has an altered starch composition,[2] and on two GM maize events (MON95275 and MON87411) with a suppression cassette that confers tolerance to certain coleopteran pests.[3,4]

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 chance that the event could establish itself or if gene flow to other species might occur.

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[5,6] 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.[7] Hybridisation of GM maize with other species than teosinte, the wild relative of maize, cannot occur.

Maize does not tolerate prolonged cold and frost,[8] and requires warm conditions in order to grow.[7,9] In cultivation areas with warm climatic conditions, volunteers – maize not deliberately planted – may 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.[7]

Maize is very sensitive to weed competition.[10] During the long process of domestication, maize has lost the ability to persist in the wild.[6] A soil seed bank, small seeds, and an extended period of flowering and seed production are characteristics often observed in persistent weeds.[11] Maize lacks all these characteristics. After ripening, the seeds (the kernels) adhere to the cob and do not scatter naturally.[7,12] 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.[13,14] 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. Maize can hybridise with teosinte, the wild relative of maize. However, as teosinte is absent in maize fields and in nature in the Netherlands,[8] hybridisation of GM maize with teosinte will not occur in the Netherlands.

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 MON94804 was produced by *A. tumefaciens* mediated transformation of mature maize HCL301 seed embryo explants. The disarmed *A. tumefaciens* ABI strain and plasmid PV-ZMAP527892, which contains a single T-DNA with the *GA20ox_SUP* suppression cassette and the *cp4 epsps* selectable marker cassette which is flanked by *loxP* sites, were used. Glyphosate was applied to inhibit growth of untransformed plant cells. PCR assays were subsequently used to select plants in which the T-DNA was present and plasmid vector backbone sequences were absent. These plants were crossed with a Cre recombinase expressing maize line, which was developed using plasmid PV-ZMOO513642. The Cre recombinase enzyme excises DNA sequences which are present between *loxP* sites. The progeny of the two maize lines produces Cre recombinase resulting in the excision of the *cp4 epsps* selectable marker cassette, and one of the *loxP* sites. Conventional breeding and subsequent selection was used to obtain plants with the *GA20ox_SUP* suppression cassette, but without the *cre* gene (and any other sequences from the PV-ZMOO513642 plasmid).

A description of the genetic elements inserted in MON94804 is listed in the table below. The list is limited to the introduced expression and suppression cassettes, and corresponding traits, as well as regulatory elements (promoters and terminators).

Introduced cassettes	**Regulatory elements**	**Encoded products and traits**
GA20ox_SUP The *GA20ox_SUP* suppression cassette consists of an inverted repeat derived from maize GA20ox3 and GA20ox5 coding sequences, flanked by three Osa-miR1425 fragments from rice (*Oryza sativa)*.	Promoter and leader from the *Rice tungro bacilliform virus* (RTBV). This promoter is primarily active in the vascular bundles of maize and has a relatively low activity in reproductive tissues.[19] Intron and flanking exon sequence from the maize heat shock protein 70 (*hsp70*) gene	The *GA20ox_SUP* suppression cassette generates GA20ox_SUP miRNA which is processed to a 21-nucleotide miRNA that is recognised by the endogenous RNAi machinery resulting in the down-regulation of gene expression of gibberellic acid 20 oxidase genes *ZmGA20ox3* and *ZmGA20ox5*. The *GA20ox_SUP* suppression cassette leads to a reduction of gibberellic acid/ gibberellin levels resulting in a reduction of internode length and reduced overall plant height.[15]

	Synthetic 3'UTR (GST43) based on multiple 3'UTR sequences from maize	
See references for detailed descriptions of the traits.		

3.4 Molecular characterisation

The applicant used Next Generation Sequencing (NGS) of MON94804 maize and subsequent mapping to PV-ZMAP527892 and PV-ZM00513642 plasmid sequences to determine the number of inserts and to confirm the absence of unintended plasmid sequences. Large numbers of reads mapped to the intended T-DNA (containing the *GA20ox_SUP* suppression cassette, but not the *cp4 epsps* selectable marker cassette). A few reads mapped to the PV-ZMAP527892 backbone, the selectable marker cassette and to PV-ZM00513642 sequences. According to the applicant these reads are likely caused by environmental bacteria that were present in the tissue from which the DNA was isolated.

Overall, the results indicate that MON94804 contains one copy of the intended T-DNA containing the *GA20ox_SUP* suppression cassette, and a single *loxP* site. No other unintended sequences such as backbone sequences from the PV-ZMAP527892 plasmid, the selectable marker cassette or sequences from the *cre* gene containing PV-ZM00513642 plasmid were present.

Directed sequencing (locus specific PCR and DNA sequence analyses) was used to obtain the sequence of the insert, the adjacent flanking DNA (1,000 base pairs on both sides), and the 5' and 3' insert-to-flank junctions. Sequence analysis of the insert indicated that it is 2,733 base pairs long, and confirmed that the selectable marker cassette and one of the *loxP* sites are not present in MON94804 maize. In addition, it showed that the left T-DNA border was truncated and that the right T-DNA border is not present in MON94804. The sequence of the insert is otherwise identical to the T-DNA region in PV-ZMAP527892.

The sequence of the insertion site in MON94804 was compared to the corresponding region in HCL301 maize (i.e. the conventional control) to determine whether endogenous maize genes where disrupted by the insertion. This comparison revealed that 41 base pairs were deleted, most likely by the insertion of the T-DNA. Bioinformatic analysis of the flanking regions of the insert indicated that the insert was inserted in chromosome 1 of the maize genome and that no endogenous genes were disrupted by the insertion.

The sequences of the insert and the junctions between the insert and the flanking regions were translated *in silico* (all six reading frames from stop-to-stop codon), and bioinformatically analysed using the FASTA algorithm to assess similarities to allergens, toxins and biologically active proteins (Blast E-value of $\leq 10^{-5}$). To assess similarities to allergens an eight amino acid sliding window search was done as well. The sliding window search yielded a match to an 'allergen collagen alpha-2(I) chain precursor' from *Bos taurus*. COGEM notes that the assessment of potential allergenicity is not part of the environmental risk assessment but is included in the food/feed safety assessment which is carried out by EFSA and WFSR (see paragraph 4). The FASTA search of the insert (PRT_2022 database) yielded an alignment to 'ORF P46' from the *Rice tungro bacilliform virus*. A

promoter and leader from this virus are part of the *GA20ox_SUP* suppression cassette. No other similarities to biologically active proteins or toxins were identified.

Maize has five major *ZmGA20ox* genes and four putative *ZmGA20ox* genes.[15] The GA20ox_SUP suppression cassette was designed to target *ZmGA20ox3* and *ZmGA20ox5*, which have relatively higher expression levels in vegetative tissues such as stalk and leaf and relatively lower expression levels in reproductive tissues such as pollen and grain. Bio-informatic analyses were carried out to investigate whether the mature GA20ox_SUP miRNA sequence could affect endogenous maize transcripts besides the targeted gibberellin 20-oxidase 3 and gibberellin 20-oxidase 5. No unintended targets were identified. To validate the specificity of the *GA20ox_SUP* suppression cassette, mRNA levels of *ZmGA20ox1* - the gene most homologous to *ZmGA20ox3* and *ZmGA20ox5*[15] - were analysed as well. *ZmGA20ox1* mRNA levels were not significantly different in over season leaf 1, over season root, forage, and pollen. *ZmGA20ox1* mRNA levels were higher in over season leaf 4, forage root, and reduced in silk and grain tissues. According to the applicant, the changes in *ZmGA20ox1* mRNA levels are most likely caused by a GA-dependent feedback loop that regulates the expression level of *ZmGA20ox1* in response to the change in *ZmGA20ox3* and *ZmGA20ox5* expression.

Overall, the molecular characterisation was conducted according to the criteria previously laid down by COGEM.[16]

Conclusion: The molecular characterisation of maize MON 94804 is adequate and no indications for potential environmental risks were identified.

3.5 Phenotypic and agronomic characteristics

Dormancy and germination of MON94804 seeds were analysed under optimal and suboptimal temperature regimes and compared to HC301+HCL617 maize (i.e. the conventional control). No statistically significant differences were detected.

The applicant assessed eleven phenotypic and agronomic characteristics of maize MON94804 at eight field sites in the United States of America, and compared these to those of HC301+HCL617 maize (i.e. its conventional counterpart). Differences between MON94804 and its conventional counterpart were observed for some of the assessed characteristics (i.e. days to flowering, days to maturity, lodging, moisture, final stand count), but these characteristics were within the range of the reference varieties (‘equivalent’, or ‘equivalence more likely than not’). The plant and ear height of MON94804 were smaller than those of the conventional counterpart and the reference varieties (‘non-equivalent’). MON94804 was developed with the aim to obtain maize plants with a short stature, and is thus expected to have a reduced plant height. According to the applicant, a reduced ear height is also an expected phenotypic change. In addition, the applicant states that the observed values are within the reported range of conventional maize varieties.

The response of MON94804 to abiotic stress, disease damage, and arthropod damage was assessed as well. No difference in abiotic stress response was observed except for high wind damage, which was less severe in MON94804 at two time points at one of the field sites. Reduced plant height is expected to improve tolerance to wind damage. The difference was, however, not consistently observed among the sites where responses to high winds were assessed, and the reduction in damage fell within the range of the reference varieties. No difference in disease and arthropod damage were observed, except for less severe common rust symptoms at one time point at one of the field sites. This difference was within the range of the reference varieties, and not observed consistently.

Overall, there are no indications that MON94804 has an altered survivability compared to conventional maize, and no indications that it will be able to survive or establish in the Dutch environment. Maize is a highly domesticated crop that has lost the ability to persist in the wild. The reduced plant and ear height will not give MON94804 maize an advantage under natural conditions, or alter the survivability of MON94804 maize in the Netherlands.

Conclusion: The trait introduced in GM maize MON94804 does not alter the survivability of maize 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 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 previously published several recommendations for further improvement of the GS plan,[17,18] but considers the current GS plan adequate for import and processing of maize MON94804.

6. Overall conclusion

COGEM is of the opinion that import and processing of maize MON94804 (a hypothetical case, as GM maize MON94804 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

COGEM notes that although an application for import and processing of MON94804 was filed, the applicant states that it will not be commercialised as a single event and will only be used as a parental line 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.[19] COGEM is of the opinion that there is no need to file an application for a single event that will never be commercialised. Potential risks of the single events used to create a stacked event can be assessed when the stacked event is assessed.

COGEM considers the assessment of single events which will not be commercialised as stand-alone products superfluous and considers the request for authorisation of MON94804 for import and processing and use in food and feed an example of following unnecessary procedures. These procedures result in unnecessary delays in the assessment of stacked GM events.

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