Second renewal of the authorisation for import and processing of genetically modified maize MON810

COGEM advice CGM/230512-01

- The present application (GMFF-2022-9450) concerns the second renewal of the authorisation for import and processing for use in feed and food (including pollen) of genetically modified (GM) maize MON810;
- GM maize MON810 has been authorised for import and processing for use in feed and food since 1998. The use of MON810 pollen in food has been authorised in 2013;
- MON810 expresses the *cry1Ab* gene, resulting in tolerance to certain lepidopteran pests;
- 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 nature, therefore hybridisation of GM maize with other species is not possible;
- The updated bioinformatic analyses indicate that ~13 Mb of chromosome 5 was deleted by the insertion of the *cry1Ab* insert;
- The literature review and post-market environmental monitoring reports do not give any indication that MON810 poses a risk to the environment;
- COGEM is of the opinion that import and processing of maize MON810 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-2022-9450), filed by Bayer Agriculture BV, concerns the second renewal of the authorisation for food (including pollen), feed, import and processing of genetically modified (GM) maize MON810. MON810 expresses the *cry1Ab* gene, resulting in tolerance to certain lepidopteran pests. It was first authorised for food, feed, import and processing and cultivation in Europe in 1998.[1] As market authorisations remain valid for ten years, a request to renew the authorisation was submitted in 2007. The authorisation for food, feed, import and processing was renewed in 2017.[2] In 2013, the use of MON810 pollen in food was authorised as well.[3] The renewal of the authorisation for cultivation is still pending.

The applicant recently submitted an application for a second renewal of the authorisation for food (including pollen), feed, import and processing. This application contains, amongst others, monitoring reports, updated bioinformatic analyses, and a systematic literature search.

2. Previous COGEM advice

COGEM has issued several opinions on potential environmental risks of cultivation, import and processing of MON810 and concluded that it poses a negligible risk to the environment.[4,5,6] In addition, COGEM has advised on import and processing of several stacked maize events containing MON810, such as MON88017xMON810[7], DP4114xMON810xMIR604xNK603[8] and NK603x MON810.[9]

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[10,11] 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.[12] Hybridisation of GM maize with other species than teosinte, the wild relative of maize, cannot occur.

Maize does not tolerate prolonged cold and frost,[13] and requires warm conditions in order to grow.[12,14] In cultivation areas with warm climatic conditions, volunteers – i.e. 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.[12]

Maize is very sensitive to weed competition.[15] During the long process of domestication, maize has lost the ability to persist in the wild.[11] A soil seed bank, small seeds, and an extended period of flowering and seed production are characteristics often observed in persistent weeds.[16] Maize lacks all these characteristics. After ripening, the seeds (the kernels) adhere to the cob and do not scatter naturally.[12,17] 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.[18,19] Any volunteers emerging will be killed by frost at the onset of winter.[13] 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,[13] 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 gene, trait and regulatory element

MON810 was created using microparticle bombardment. The *cry1Ab* insert in MON810 maize is truncated and lacks the *nos* terminator and part of the e35S promoter.[5] The insertion disrupted a E3 ubiquitin ligase gene located at the 3'end of the insert and led to rearrangements in the flanking regions.[5] It was previously reported that MON810 produces chimeric transcripts consisting of *cry1Ab* sequences and E3 ubiquitin ligase sequences (in an antisense orientation). *In silico* translation indicated that these correspond to putative fusion products with 2 and 18 amino acids in addition to the Cry1Ab protein.[5]

Descriptions of the inserted genetic elements in MON810 are 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 products**	**Traits**	**Regulatory elements**
cry1Ab	Cry1Ab protein derived from *B. thuringiensis* subsp. *kurstaki* [20,21]	Resistance to certain lepidopteran insects	Enhanced *35S* (*e35S*) promoter from *Cauliflower mosaic virus* (CaMV) and Zmhsp70 intron sequences from *Zea mays*; terminator is absent
For a detailed description of the introduced genes and traits, see references			

3.4 Updated bioinformatic analyses

The applicant updated the bioinformatic analyses using databases assembled in January 2021. The *cry1Ab* insert and the junctions with its 5' and 3' flanking regions were bioinformatically analysed from stop to stop codon in all six potential reading frames. The results of the bioinformatic analyses with the updated allergen, gliadin and glutenin protein sequence database (AD_2021) were not assessed by COGEM, because 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). Bioinformatic analyses with the other databases (i.e. the protein sequence database (PRT_2021) and the toxin protein sequence database (TOX_2021)) identified sequences corresponding to elements of the insert, i.e. the CaMV promoter and Cry1Ab itself. The analyses also identified the E3 ubiquitin ligase gene which was disrupted by the insertion of the *cry1Ab* insert. Other similarities retrieved in the bioinformatic analyses were of low sequence identity and required gaps to optimise the alignment.

The flanking regions of the *cry1Ab* insert were bioinformatically analysed using the Zm-B73 genome in GenBank (ZMA_2021) to check whether the insertion of the *cry1Ab* insert disrupted any endogenous genes. The results indicated that the insert was integrated in chromosome 5 of the maize genome and confirmed the previously reported disruption of a E3 ubiquitin ligase gene at the 3'end of the insert.[5] Mapping of the 5' and 3' flanking sequences of the insert to the Zm-B73 maize genome indicated that ~13 Mb of chromosome 5 was deleted by the insertion. According to the applicant, 280 annotated genes are present in the deleted region. COGEM identified two scientific publications

that studied gene expression of MON810.[22,23] According to these publications there are some indications that MON810 might have slightly delayed maturation processes,[22] and is more sensitive to drought stress in the first few days.[23] The loss of ~13 Mb on chromosome 5 does, however, not seem to interfere with the growth and vigor of MON810, as it has been cultivated successfully for at least 25 years.

COGEM is of the opinion that the molecular characterisation of MON810 maize was performed correctly and meets the requirements of COGEM.[24]

Conclusion: The bioinformatic analyses of maize MON810 have been updated and performed adequately. No indications for potential environmental risks were identified.

3.5 Systematic literature search and unpublished studies

The systematic literature search, which was submitted as part of the renewal application, covered the period from January 2012 to July 2022 and addressed the question "Does MON 810 maize derived food/feed products and the introduced insect protection trait have adverse effects on human and animal health and the environment?" Eighty publications were retrieved. According to the applicant, these publications did not report any new hazards, modified exposure, or new scientific uncertainty of MON810 and therefore did not have any implication for its risk assessment of MON810.

Conclusion: The systematic literature search did not provide any indications that import of MON810 maize poses an environmental risk.

3.6 Annual monitoring reports

The applicant supplied annual reports on the post-market environmental monitoring (PMEM) carried out between July 2016 and June 2021. These reports contain amongst others information on annual literature searches carried out by the applicant, and on the monitoring which is carried out by operators involved in import, handling and processing of viable GM maize. These operators are members of the European trade associations COCERAL, UNISTOCK or FEDIOL. They should report any occurrence of unanticipated adverse effects arising from MON810 maize, including adventitious populations resisting routine eradication procedures, to these trade associations.

According to the monitoring reports, no relevant publications that invalidate the initial conclusions on the risk assessment of MON810 were identified in the annual literature search, and no adverse health or environmental effects were reported by the trade associations involved in the monitoring of import and processing of MON810.

The PMEM of import and processing carried out between 2016 and 2021 did not provide any indications that import and processing of MON810 poses a risk to the environment.

Conclusion: The information in the annual monitoring reports gives no indication of adverse effects or incidents resulting from import and/or processing of MON810 maize.

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 did not propose any changes to the existing post-market environmental monitoring (PMEM) plan for maize line MON810. COGEM has published several recommendations for further improvement of the general surveillance (GS) plan,[25,26] which is part of a PMEM plan, but considers the current GS (and PMEM) plan adequate for import and processing of maize MON810.

6. Overall conclusion

The application for the second renewal of the authorisation for food (including pollen), feed, import and processing of GM maize MON810 contains, amongst others, updated bioinformatic analyses, a systematic literature search, and reports on the post-market environmental monitoring that was carried out. The updated bioinformatic analyses indicated that ~13 Mb of chromosome 5 was lost by the insertion of the *cry1Ab* insert. This, however, does not seem to interfere with the growth and vigor of MON810, as it has been cultivated successfully for at least 25 years. The systematic literature search, the PMEM of import and processing carried out between 2016 and 2021, and the PMEM of cultivation carried out between 2006 and 2016[27] do not provide indications that MON810 poses a risk to the environment.

Overall, COGEM is of the opinion that import and processing of maize MON810 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.

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