Import and processing of genetically modified cotton GHB811

COGEM advice CGM/190401-01

- The present application (EFSA/GMO/ES/2018/154) concerns the authorisation for import and processing for use in feed and food of genetically modified (GM) cotton (*Gossypium hirsutum*) GHB811;
- GM cotton GHB811 expresses the *2mepsps* and *hppdPfW336-1Pa* genes, which confer tolerance to glyphosate and hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor containing herbicides, respectively;

- In the Netherlands, cultivation of cotton is not possible and feral cotton populations do not occur;
- Wild relatives of cotton are not present in the Netherlands, therefore hybridisation with other species is not possible;

- The molecular characterisation of GM cotton GHB811 meets the criteria of COGEM;
- There are no indications that the introduced traits alter the fitness of GM cotton GHB811;
- There is no reason to assume that the introduced traits will allow GM cotton GHB811 to survive in the Dutch environment;

- COGEM is of the opinion that import and processing of GM cotton GHB811 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 (EFSA/GMO/ES/2018/154), filed by BASF, concerns import and processing of genetically modified (GM) cotton (*Gossypium hirsutum*) GHB811. This GM cotton line contains the *2mepsps* and *hppdPfW336-1Pa* genes, conferring tolerance to glyphosate and hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor containing herbicides, such as isoxaflutole. Since 2011 and 2016, crops with the same introduced traits (GM cotton (*G. hirsutum*) GHB614 and GM soybean FG72, respectively), are authorised for import and processing in the European Union.[1,2]

2. Previous COGEM advice

COGEM has not previously advised on import and processing of GM cotton GHB811. COGEM did advise positively on GM cotton line GHB614, which contains the gene *2mepsps*.[3,4]

Furthermore, COGEM advised positively on GM soybean line FG72, which contains the genes *2mepsps* and *hppdPfW336*.[5]

3. Environmental risk assessment

3.1 Characteristics of cotton

Cotton is a member of the genus *Gossypium* and belongs to the *Malvaceae* family. The majority of cultivated cotton is *Gossypium hirsutum* (90%), followed by *Gossypium barbadense* (5%), and *Gossypium arboreum* and *Gossypium herbaceum* (together ≤ 5%).[6,7,8] The only cultivated cotton species in Europe is *G. hirsutum*, which is grown in Greece, Spain and Bulgaria.[9,10]

Cotton requires at least 500 mm of rainfall during the growing season, or can be grown as irrigated crop.[7] Cotton is highly sensitive to temperature, and susceptible to frost.[7,8,11,12] Seed germination and plant development cease below a temperature of 12ºC and delay when the temperature rises above 38ºC.[7,8] The optimal daytime temperature for *G. hirsutum* ranges between 30 and 35ºC.[7,12] *G. hirsutum* requires 180 to 200 frost-free days of uniformly high temperatures (averaging 21-22ºC) after planting.[8,13] From the time of planting to 60% boll opening (i.e., when seed is mature), a minimum of 2050 day degrees* is required.[8,14] In the Netherlands, there are on average 85 days with a daily maximum temperature of ≥ 20ºC per year.[15] Frost days in the Netherlands generally occur from October up to and including April,[16] and it is not uncommon for frost days to occur in early summer. In the summer (June, July and August), when temperatures are highest, the daily temperature averages 17ºC.[17] This corresponds to an accumulated average of 436 day degrees. In the remaining months, the temperature is insufficiently high to reach the accumulated amount of day degrees required (2050) for the growth and maturation of cotton. Considering the above, the Dutch climate conditions are unsuitable for the life cycle of cotton.

Cotton plants reproduce sexually.[8] Cotton is predominantly a self-pollinating species, but cross-pollination may occur.[18] Dissemination of pollen by wind is (almost) absent.[7,8] Outcrossing rates for cotton are strongly influenced by the presence of insects. Cotton seeds can remain dormant for 2-3 months, but this trait is reduced or eliminated by selective breeding. Seeds from modern cotton cultivars do not possess dormancy.[7] Cotton seeds from cultivars usually do not survive in humid soil and the formation of seed banks is unlikely.[8,18] Seedlings are also sensitive to competition from weeds.[7]

Cotton volunteers occur in areas where cotton is cultivated and may occur due to spilling during transport or when feeding cattle.[8] There are reports that *G. hirsutum* and *G. herbaceum* are naturalised in some Southern European countries, e.g. Greece and Spain.[19,20] COGEM is not aware of any reports on feral cotton populations in Northwestern Europe. Wild relatives of cotton

* Day degrees (or heat units) are a measure of time and temperature required to reach a certain plant developmental stage. They are calculated based on the daily minimum and maximum temperature minus the threshold temperature for growth and development of cotton (12ºC): [(daily max. temperature - 12) + (daily min. temperature - 12)] / 2. The day degrees for each day are summed during the growing season. When the average daily temperature drops below the threshold temperature, the daily increment of day degrees is set to zero.

(*Gossypium* spp.) do not occur in Northwestern Europe. Therefore, hybridisation with wild relatives cannot occur in Northwestern Europe.[7]

Conclusion: The Dutch climate is unsuited for cotton cultivation. In the Netherlands, feral cotton populations do not occur, and hybridisation with other species is not possible because no wild relatives of cotton are present.

3.2 Description of the introduced genes and traits

GHB811 was developed using *Agrobacterium tumefaciens* mediated transformation. 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**
hppdPfW336-1Pa	Modified 4-hydroxy-phenylpyruvate dioxygenase (HPPD W336) from *Pseudomonas fluorescens*[21]	Tolerance to hydroxy-phenylpyruvate dioxygenase inhibitor containing herbicides	Pcsvmv promoter from *Cassava vein mosaic virus* and ThistonAt H4 terminator sequence of *Arabidopsis thaliana*
2mepsps	Modified 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) originating from *Zea mays*[22]	Tolerance to glyphosate containing herbicides	Ph4a748 sequence including promoter region of the histon H4 gene and ThistonAt H4 terminator sequence from *A. thaliana*
For a detailed description of the introduced genes and traits, see references			

3.3 Molecular characterisation

Southern blot analyses show that cotton GHB811 contains one copy of the insert at a single integration locus, and demonstrate the absence of backbone sequences.

The applicant determined the sequence of the GHB811 insert and adjacent flanking sequences. During integration of the T-DNA, one deletion occurred. A fragment of 13 bp was deleted from the cotton genome. According to the applicant, no endogenous gene or regulatory element has been disrupted at the insertion site.

The applicant screened the junctions between the T-DNA insert and the flanking cotton genomic DNA as well as the entire insert (from stop to stop codon) for potential newly created open reading frames (ORFs). According to the applicant, no significant sequence similarities between the putative products of these ORFs and no known or putative allergenic or toxic proteins were detected in bioinformatics analyses.

The molecular characterisation was conducted according to the criteria previously laid down by COGEM.[23]

Conclusion: The molecular characterisation of cotton GHB811 is adequate and no indications for potential environmental risks were identified.

3.4 Phenotypic and agronomic characteristics

The applicant analysed the phenotypic and agronomic characteristics of cotton GHB811 and noted that these, except for the introduced traits, are not different from the non-GM near-isogenic control line, and are equivalent to the reference varieties, taking into account natural variation. The introduced traits do not give reason to assume that GHB811 has an altered fitness compared to conventional *G. hirsutum*. COGEM is of the opinion that there are no indications that cotton GHB811 will be able to survive or establish in the Dutch environment.

Conclusion: There are no indications that the introduced traits allow the event GM cotton GHB811 to survive in the Netherlands. GHB811 does not have an 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, RIKILT carries out a food and/or feed assessment for Regulation (EC) 1829/2003 applications. The outcome of the assessment by other organisations (EFSA, RIKILT) was not known when this advice was completed.

5. Post-market environmental monitoring (PMEM)

The applicant supplied a post-market environmental monitoring (PMEM) plan. COGEM has published several recommendations for further improvement of the general surveillance (GS) plan[24,25] but considers the current GS plan adequate for import and processing of cotton GHB811.

6. Overall conclusion

COGEM is of the opinion that import and processing of cotton (*G. hirsutum*) GHB811 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.

References

1. Commission Decision of 17 June 2011 authorising the placing on the market of products containing, consisting of, or produced from genetically modified cotton GHB614 (BCS-GHØØ2-5) pursuant to Regulation (EC) No 1829/2003 of the European Parliament and of the Council. 18.6.2011 L 160/90

2. Commission Implementing Decision (EU) 2016/1215 of 22 July 2016 authorising the placing on the market of products containing, consisting of, or produced from genetically modified soybean FG72 (MST-FGØ72-2) pursuant to Regulation (EC) No 1829/2003 of the European Parliament and of the Council. 26.7.2016 L 199/16
3. COGEM (2008). Import and processing of genetically modified cotton GHB614. COGEM advice CGM/080509-01
4. COGEM (2013). Cultivation of glyphosate tolerant GHB614 cotton. COGEM advice CGM/130321-01
5. COGEM (2012). Import of genetically modified soybean FG72 with glyphosate and isoxaflutole herbicide tolerance. COGEM advice CGM/120104-02
6. Crop Protection Compendium (2007). *Gossypium* and *Gossypium hirsutum* (cotton). CD-ROM edition, © Cab International 2007, Nosworthy way, Wallingford, United Kingdom
7. The Organisation for Economic Co-operation and Development (2008). Consensus document on the biology of cotton (*Gossypium* spp.)
8. Office of the Gene Technology Regulator (2016). The biology of *Gossypium hirsutum* L. and *Gossypium barbadense* L. (cotton)
9. European Commission (2016). Agricultural and rural development. https://ec.europa.eu/agriculture/cotton_en. (visited: March 15th, 2019)
10. Rüdelsheim PLJ & Smets G (2012). Baseline information on agricultural practices in the EU. Cotton (*Gossypium hirsutum* L.). www.europabio.org/agricultural-biotech/publications/agronomic-practices-cotton-europe (visited: March 15th, 2019)
11. Unruh BL & Silvertooth JC (1997). Planting and irrigation termination timing effects on the yield of Upland and Pima cotton. J. Product. Agricult. 10: 74-79
12. Reddy KR *et al.* (1992). Temperature effects on early season cotton growth and development. Agron. J. 84: 229-237
13. Duke JA (1983). *Gossypium hirsutum* L. Handbook of Energy Crops. unpublished. https://hort.purdue.edu/newcrop/duke_energy/Gossypium_hirsutum.html (visited: March 15th, 2019)
14. Ritchie GL *et al.* (2007). Cotton growth and development, rev. ed. University of Georgia Cooperative Extension Bulletin 1252: 1-16
15. Koninklijk Nederlands Meteorologisch Instituut (KNMI). Uitleg over warme dagen. www.knmi.nl/kennis-en-datacentrum/uitleg/warme-dagen (visited: March 15th, 2019)
16. Koninklijk Nederlands Meteorologisch Instituut (KNMI). Vorstdagen. www.knmi.nl/kennis-en-datacentrum/uitleg/vorstdagen (visited: March 15th, 2019)
17. Koninklijk Nederlands Meteorologisch Instituut (KNMI). Uitleg over zomer. www.knmi.nl/kennis-en-datacentrum/uitleg/zomer (visited: March 15th, 2019)
18. Andersson MS & 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
19. Polunin O (2005). Flowers of Greece and the Balkan – a field guide. Oxford University Press Inc., New York
20. Tutin TG *et al.* (2005). Flora Europeae. Volume 2. Rosaceae to Umbelliferae. Cambridge University Press, United Kingdom

21. Boudec P *et al*. (2001). Mutated hydroxyphenylpyruvtate dioxybenase, DNA sequence and isolation of plants which contain such a gene and which are tolerant to herbicides. United States Patent
22. Lebrun M *et al*. (1997). Mutated 5-enol pyruvylshikimate-3-phosphate synthase gene coding for said protein and transformed plants containing said gene. Organisation Mondiale de la Propriété Intellectuelle
23. COGEM (2014). Reconsideration of the molecular characterisation criteria for marketing authorisation of GM crops. COGEM report CGM/140929-02
24. COGEM (2010). General Surveillance. COGEM report CGM/100226-01
25. COGEM (2015). Advice on improving the general surveillance of GM crops. COGEM advice CGM/150601-02