Import and processing of genetically modified cotton DAS-81910-7

COGEM advice CGM/170717-02

- The present application (EFSA/GMO/NL/2016/136) concerns the authorisation for import and processing for use in feed and food of genetically modified (GM) cotton (*Gossypium hirsutum*) DAS-81910-7;
- GM cotton DAS-81910-7 expresses the *pat* and *aad-12* genes, which confer tolerance to aryloxyalkanoate based (such as 2,4-D) or glufosinate-ammonium containing herbicides.

- 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, so hybridisation with other species is not possible;

- The molecular characterisation of cotton DAS-81910-7 meets the criteria of COGEM;
- There are no indications that the introduced traits alter the fitness of cotton DAS-81910-7;
- There is no reason to assume that the introduced traits will allow GM cotton DAS-81910-7 to survive in the Dutch environment;

- COGEM is of the opinion that import and processing of cotton DAS-81910-7 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/NL/2016/136), filed by Dow AgroSciences LLC., concerns import and processing of genetically modified (GM) cotton (*Gossypium hirsutum*) DAS-81910-7. The GM cotton line contains the *pat* and *aad-12* genes, conferring tolerance to aryloxyalkanoate based (such as 2,4-D) and glufosinate-ammonium containing herbicides.

2. Previous COGEM advice

COGEM did not previously advise on import and processing of GM cotton DAS-81910-7. COGEM did advise positively on crops containing the same genes (*aad-12* and *pat*) as GM cotton DAS-81910-7, such as GM soybean lines DAS-68416-4 and DAS-44406-6.[1,2] COGEM also advised positively on other GM cotton lines, such as GHB614xT304-40xGHB119, GHB614xLLCotton25xMON15985 and LLCotton25xMON15985.[3,4]

3. Environmental risk assessment

3.1 Aspects of the wild-type crop

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%).[5,6,7] The only cultivated cotton species in Europe is *G. hirsutum*, which is grown in Greece, Spain and Bulgaria.[8,9]

Cotton requires at least 500 mm of rainfall during the growing season, but can also be grown as irrigated crop.[6] Cotton is highly sensitive to temperature, and susceptible to frost.[6,7,10,11] Seed germination and plant development cease below a temperature of 12 ºC and delay when the temperature rises above 38 ºC.[6,7] The optimal daytime temperature for *G. hirsutum* ranges between 30 and 35 ºC.[6,11] *G. hirsutum* requires 180 to 200 frost-free days of uniformly high temperatures (averaging 21-22 ºC) after planting.[7,12] From planting of cotton to 60% boll opening (i.e., when seed is mature), a minimum of 2050 day degrees* is required.[7,13] In the Netherlands, there are on average 85 days with a daily maximum temperature of ≥ 20 ºC per year.[14] Frost days in the Netherlands generally occur from October up to and including April[15], 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 17 ºC.[16,17] 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.[7] Cotton is predominantly a self-pollinating species, but cross-pollination may occur.[18] Dissemination of pollen by wind is (almost) absent.[6,7] 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.[6] Cotton seeds from cultivars usually do not survive in humid soil and the formation of seed banks is unlikely, as seeds become weathered when they do not germinate directly.[7,18] Seedlings are also sensitive to competition from weeds.[6]

Cotton volunteers occur in areas where cotton is cultivated and may occur due to spilling during transport or when feeding cattle.[7] 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 (*Gossypium* spp.) do not occur in Northwestern Europe. Therefore, hybridisation with wild relatives cannot occur in Northwestern Europe.[6]

* 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 (12 ºC): [(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.

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

Cotton DAS-81910-7 was developed using *Agrobacterium tumefaciens* mediated transformation using the binary vector pDAB4468.

Introduced genes	Encoded proteins (enzymes)	Traits
pat	Variant of phosphininothricin N-acetyltransferase (PAT) originating from *Streptomyces viridochromogenes*[1,2,21,22]	Tolerance to glufosinate-ammonium containing herbicides
aad-12	Aryloxyalkanoate dioxygenase-12 (AAD-12) enzyme originating from *Delftia acidovorans*[1,2,23]	Tolerance to aryloxyalkanoate based herbicides, such as 2,4-D
For a detailed description of the introduced genes and traits, see references		

3.3 Molecular characterisation

Southern blot analyses show that cotton DAS-81910-7 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 cotton DAS-81910-7 insert and adjacent flanking sequences. According to the applicant, no endogenous gene or regulatory element was disrupted at the insertion site.

The applicant also 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). The applicant stated that no significant sequence similarities with toxic proteins were detected in these bioinformatic analyses, using a non-redundant protein sequence database.

The molecular characterisation was conducted according to the criteria previously laid down by COGEM.[24]

Conclusion: The molecular characterisation of cotton DAS-81910-7 is adequate. There are no indications that expression of the introduced traits or any combination thereof will alter the fitness of cotton DAS-81910-7.

3.4 Phenotypic and agronomic characteristics

The applicant analysed the phenotypic and agronomic characteristics of cotton DAS-81910-7. The introduced traits do not give reason to assume that DAS-81910-7 has an altered fitness compared to conventional cotton. According to the applicant, no meaningful biological changes to agronomic

performance are associated with DAS-81910-7 compared to conventional cotton. COGEM is of the opinion that there are no indications that cotton DAS-81910-7 will be able to survive or establish in the Dutch environment.

Conclusion: Cotton DAS-81910-7 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 new post-market environmental monitoring (PMEM) plan. COGEM has published several recommendations for further improvement of the general surveillance (GS) plan[25,26] but considers the current GS plan adequate for import and processing of cotton DAS-81910-7.

6. Additional remark

The dossier contained a discrepancy concerning expression levels of the PAT protein. The applicant claimed that PAT protein was not detected in control tissues. However, in the provided reference it is stated that detectable levels of PAT protein were found in a small number of control samples. According to the authors of this report, this was most likely due to a sampling error and/or contamination. The results of these analyses do not give reasons to assume that cotton DAS-81910-7 could pose an environmental risk. COGEM is of the opinion that this incongruence does not affect the outcome of the environmental risk assessment.

7. Overall conclusion

COGEM is of the opinion that import and processing of cotton DAS-81910-7 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. COGEM (2011). Import of genetically modified soybean DAS-68416-4 with two herbicide tolerance traits. COGEM advise CGM/111114-02

2. COGEM (2013). Import of genetically modified soybean DAS-44406-6 with three herbicide tolerance traits. COGEM advise CGM/130627-01
3. COGEM (2016). Import and processing of genetically modified cotton GHB614 x T304-40 x GHB119. COGEM advice CGM/161124-01
4. COGEM (2015). Import and processing of genetically modified cotton GHB614xLLCotton25xMON15985 and LLCotton25xMON15985. COGEM advice CGM/151008-01
5. Crop Protection Compendium (2007). *Gossypium* and *Gossypium hirsutum* (cotton). CD-ROM edition, © Cab International 2007, Nosworthy way, Wallingford, United Kingdom
6. The Organisation for Economic Co-operation and Development (2008). Consensus document on the biology of cotton (*Gossypium* spp.)
7. Office of the Gene Technology Regulator (2016). The biology of *Gossypium hirsutum* L. and *Gossypium barbadense* L. (cotton)
8. European Commission (2016). Agricultural and rural development. https://ec.europa.eu/agriculture/cotton_en. (visited: June 13th, 2017)
9. Rüdelsheim PLJ & Smets G (2012). Baseline information on agricultural practices in the EU. Cotton (*Gossypium hirsutum* L.). http://www.europabio.org/agricultural-biotech/publications/agronomic-practices-cotton-europe (visited: June 13th, 2017)
10. Unruh BL & Silvertooth JC (1997). Planting and irrigation termination timing effects on the yield of Upland and Pima cotton. Journal of Production Agriculture 10: 74-79
11. Reddy KR et al. (1992). Temperature effects on early season cotton growth and development. Agron. J. 84: 229-237
12. Duke JA (1983). *Gossypium hirsutum* L. Handbook of Energy Crops. unpublished. https://hort.purdue.edu/newcrop/duke_energy/Gossypium_hirsutum.html#Cultivation (bezocht: 5 juli 2017)
13. Ritchie GL *et al.* (2007). Cotton Growth and Development, rev. ed. University of Georgia Cooperative Extension Bulletin 1252: 1-16
14. Koninklijk Nederlands Meteorologisch Instituut (KNMI). Uitleg over warme dagen. https://www.knmi.nl/kennis-en-datacentrum/uitleg/warme-dagen (bezocht: 5 juli 2017)
15. Koninklijk Nederlands Meteorologisch Instituut (KNMI). Vorstdagen. https://www.knmi.nl/kennis-en-datacentrum/uitleg/vorstdagen (bezocht: 12 juli 2017)
16. Koninklijk Nederlands Meteorologisch Instituut (KNMI). Uitleg over zomer. https://www.knmi.nl/kennis-en-datacentrum/uitleg/zomer (bezocht: 13 juli 2017)
17. Koninklijk Nederlands Meteorologisch Instituut (KNMI). Uitleg over warme dagen. https://www.knmi.nl/kennis-en-datacentrum/uitleg/warme-dagen (bezocht: 5 juli 2017)
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. 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
22. Wohlleben W *et al*. (1988). Nucleotide sequence of the phosphinothricin N-acetyltransferase gene form *Streptomyces viridochromogenes* Tü494 and its expression in *Nicotoana tabacum*. Gene 70: 25-37
23. Wright TR et al. (2010). Robust crop resistance to broadleaf and grass herbicides provided by aryloxyalkanoate dioxygenase transgenes. Proc. Natl. Acad. Sci. USA 107:20240-20245
24. COGEM (2014). Signalering heroverweging van de criteria voor de moleculaire karakterisering bij markttoelatingen van gg-gewassen COGEM signalering CGM/140929-02
25. COGEM (2010). General Surveillance. COGEM report CGM/100226-01
26. COGEM (2015). Advice on improving the general surveillance of GM crops. COGEM advice CGM/150601-02