Import of insect resistant and herbicide tolerant GHB119 cotton

COGEM advice CGM/120123-01

Summary

The present application by Bayer CropScience (file EFSA/GMO/NL/2011/96) concerns the import and processing for use in feed and food of cotton GHB119. Cultivation is not part of this application.

Cotton line GHB119 was produced by Agrobacterium tumefaciens *mediated transformation of conventional cotton. GHB119 expresses the* cry2Ae *gene and* bar *gene. As a result, GHB119 cotton is resistant to certain lepidopteran pests and tolerant to glufosinate ammonium based herbicides. GHB119 has not been previously assessed by COGEM.*

In North West Europe, no wild relatives of cotton are present. Modern cotton cultivars do not possess any of the attributes commonly associated with problematic weeds. COGEM does not know of reasons to assume that the introduced traits will increase the potential of cotton to establish feral populations. Moreover, cotton cannot survive the climatologic conditions in North West Europe. Therefore, COGEM is of the opinion that incidental spillage of GHB119 seeds will not pose a risk to the environment in North West Europe.

The applicant showed by Southern blot analyses that one copy of the insert with the bar *and* cry2Ae *gene is integrated at a single locus. The applicant further showed that the backbone of the plasmid used for transformation is absent in GHB119. Bioinformatic analysis of the junctions of the insert and the cotton genomic DNA identified a total of eleven open reading frames. These sequences were analyzed for similarity to known toxins or allergens. No similarities were found. The molecular characterisation and provided General Surveillance plan meet the criteria of COGEM.*

In view of the above, COGEM is of the opinion that the risks for humans and the environment associated with import and processing of cotton line GHB119 are negligible. A food/feed safety assessment is carried out by other organisations. Therefore, COGEM abstains from advice on the potential risks of incidental consumption.

Introduction

The present notification (EFSA/GMO/NL/2011/96) by Bayer CropScience AG concerns import and processing of the genetically modified cotton line GHB119. This cotton line was produced by *Agrobacterium tumefaciens* mediated transformation of conventional cotton and expresses the *cry2Ae* gene from *Bacillus thuringiensis* and the *bar* gene from *Streptomyces hygroscopicus*. As a result, GHB119 cotton is resistant to certain lepidopteran pests and tolerant to glufosinate ammonium based herbicides.

Previous COGEM advice

GHB119 has not been previously assessed by COGEM. However, in the past COGEM has advised positively on the import of cotton and maize crops expressing the *cry2Ab2* gene.[1,2,3,4]

Furthermore, in 2011, COGEM advised positively on the import and processing of cotton line GHB614xLLCotton25 expressing the *bar* gene.[5]

Aspects of the crop

Cotton is a member of the genus *Gossypium* and belongs to the *Malvaceae* family. The majority of cultivated cotton (90%) is *Gossypium hirsutum,* but *Gossympium barbadense*, *Gossympium arboreum* and *Gossympium herbaceum* are cultivated as well.[6,7,8] In the south of Europe *G. hirsutum* cotton is grown in Greece, Spain and Bulgaria.[9]

Cotton plants reproduce sexually.[8] Cotton is predominantly a self-pollinating species, but crosspollination may occur. The pollen of cotton is large, heavy and somewhat sticky.[7,8] The viability of *G. hirsutum* pollen decreases rapidly after eight hours.[8] Outcrossing rates for cotton are strongly influenced by the prevalence of insects[8] and dissemination of pollen by wind is (almost) absent.[7] Amongst others bumblebees (*Bombus*), honeybees (*Apis*) and other bee species (*Anthophora, Melissodes* and *Halictus*) are pollinators of cotton flowers.[7] Wild relatives of cotton (*G. hirsutum*) do not occur in North West Europe. Therefore, hybridisation with wild relatives cannot occur in North West Europe.[7]

The climate in North West Europe is not suited for cotton growth. Cotton is highly sensitive to temperature and susceptible to frost. Temperature is the main factor to determine the geographic range in which cotton can be grown. *G. hirsutum* seeds do not germinate until the temperature reaches 15ºC[7] and plant development ceases when temperatures are below 12ºC.[8] Activity is delayed when the temperature rises above 38ºC.[7] For normal development, cotton needs an average of 150 days with temperatures between these values.[7] The optimum temperature for germination is 34ºC, for growth of seedlings 24-29ºC and for later continuous growth 34ºC. When the crop is grown at lower temperatures, the production of vegetative branches increases and the cropping period is extended. Because cotton is susceptible to frost, the whole growth period (which can range from 160 to 220 days) has to be free of frost.[6] In places where cotton is grown as a rain-fed crop the average rainfall is 800-1200 mm.[6] In areas where the rainfall is less than 500 mm a year, irrigation should be applied.[7] In the seedling stage cotton does not tolerate shady circumstances, and in later plant stages reduced light intensity affects flowering and fruiting.[6]

Cottonseed may be dispersed by wind or water but may also be spread during transport or when feeding cattle.[8] In addition, cottonseed may be transported by birds or rodents. Germination is less likely to occur in undisturbed sites than in disturbed sites.[8] Seeds from cotton cultivars do not possess dormancy[7,8] and will germinate in autumn if conditions are favourable. In addition, seeds will usually not survive in humid soil.[8] In regions with mild and dry winters, cottonseeds may overwinter and germinate in spring. Seedlings are sensitive to competition from weeds.[7]

Modern cotton cultivars do not possess any of the attributes commonly associated with problematic weeds, such as dormancy, persistence in soil banks, germination under adverse environmental conditions, rapid vegetative growth, a short life cycle, very high seed output, high seed dispersal and long-distance dispersal of seeds. Cotton volunteers occur in cotton growing areas and may occur when cottonseed is used as livestock feed. The occurrence of volunteer cotton is limited by the availability of adequate soil moisture or the occurrence of frost.[8] There are

reports that *G. hirsutum* and *G. herbaceum* cotton are naturalised in some Southern European countries, e.g. Greece and Spain.[10,11,12,13]

Molecular characterisation

Cotton GHB119 was developed by *Agrobacterium tumefaciens* mediated transformation of tissue from cotton variety Coker 312 using vector pTEM12. Glufosinate ammonium was used as the selective agent. The following elements were introduced in GHB119:

- Left border repeat; 24 bp *Cis*-acting element for T-DNA transfer from *A. tumefaciens;*
- 3'nos; Terminating signal of the *bar* gene from *A. tumefaciens;*
- *bar*; Glufosinate ammonium tolerance gene from *Streptomyces hygroscopicus;*
- PcsvmvXYZ; Promoter from *Cassava vein mosaic virus* for high level constitutive expression;
- P35S2; Promoter from *Cauliflower mosaic virus* for high level constitutive expression;
- 5'cab22L; Leader sequence for high level constitutive expression, from *Petunia hybrida;*
- TPssuAt; Plastid transit peptide from *Arabidopsis thaliana*;
- *cry2Ae*; Modified insect resistance *cry2Ae* gene from *Bacillus thuringiensis* subsp. *Dakota* 1715 with an adapted nucleotide sequence to optimize codon usage in plants*;*
- 3'35S; Terminating signal from *Cauliflower mosaic virus;*
- Right border repeat, 24 bp *Cis*-acting element for T-DNA transfer from *A. tumefaciens.*

The nucleotide sequence of *Cry2Ae* has been adapted to optimise codon usage during translation in cotton plants. The amino acid sequence of Cry2Ae protein is unaltered. The expressed Cry2Ae protein has a plastid transit peptide of 54 aa fused to its N-terminus.

Expressed proteins

The *cry2Ae* gene encodes a δ-endotoxin which is effective in controlling certain lepidopteran larvae, including cotton bollworm larvae (*Helicoverpa zea*) and tobacco budworm larvae (*Heliothis virescens*), which are common pests of cotton.

Upon ingestion by susceptible larvae, the Cry2Ae protein is solubilised in the midgut and activated by midgut proteases to release a toxin fragment. This toxin fragment binds to specific receptors on the epithelial surface of the midgut and causes pores to open. This leads to disruption of the movement of solutes across the gut epithelium and causes the influx of water leading to cell swelling and lysis. The gut contents are released into the body cavity allowing bacteria to breed ultimately leading to septicaemia and death.[14,15]

GHB119 is also tolerant to herbicides containing glufosinate ammonium. In non-transgenic plants glufosinate ammonium inhibits the activity of glutamine synthetase, an enzyme necessary for the production of glutamine and for ammonia detoxification.[16] The application of glufosinate ammonium leads to reduced glutamine and increased ammonia levels in non-transgenic plants.[16] Photosynthesis is inhibited and eventually the plant dies.[17] GHB119 expresses the *bar* gene which

encodes phosphinothricin-N-acetyl transferase (PAT). This protein acetylates L-phosphinothricin, the active isomer of glufosinate ammonium. The resulting compound N-acetyl-L-phosphinothricin does not inhibit the activity of glutamine synthetase.[16] As a result GHB119 is tolerant to L-phosphinothricin and thus to herbicides containing glufosinate ammonium.

Molecular analysis

The applicant demonstrated by Southern blot, PCR and sequence analysis that GHB119 cotton contains one intact gene cassette. The applicant further showed that the DNA is integrated at a single locus and that GHB119 does not contain backbone sequences of plasmid pTEM12.

The applicant determined the sequence of the integration site. A comparison with the sequence after the T-DNA insertion revealed an eight bp deletion in GHB119. The 5' and the 3' flanking sequences of the integrated DNA are of cotton origin. No unknown sequences were found.

The junctions between the T-DNA insert and the flanking plant genomic DNA were sequenced and screened for potential newly created open reading frames (ORFs). The ORFs were defined as sequences between two stop codons with a minimal size of three amino acids. In total, eleven ORFs were identified. The amino acid sequences of these ORFs were compared with known allergens in the AllergenOnline database and known toxins in several protein databases e.g. NCBI databases. These analyses showed no biologically significant sequence similarities with known toxins and allergens.

In view of the above, COGEM is of the opinion that the molecular characterisation of GHB119 has been adequately performed and meets the criteria laid down by COGEM.[18]

Environmental risk assessment

Cotton is predominantly a self-pollinating species, but crosspollination may occur. Wild relatives of cotton (*G. hirsutum*) do not occur in North West Europe. Therefore, hybridisation with wild relatives cannot occur in North West Europe.[7]

Cotton plants are very sensitive to temperature. As mentioned before, a reasonably high temperature (an average of 150 days with a temperature between 15 and 38˚C) is required in all stages of development. The optimum temperature for germination is 34ºC, for growth of seedlings 24-29ºC and for later continuous growth 34ºC. In areas where the rainfall is less than 500 mm a year, irrigation should be applied for cotton growth. In the Netherlands, May, June, July, August and September have average monthly temperatures above 12ºC, but below 18ºC.[19] In addition, in May, June, July, August and September the average monthly precipitation does not exceed the 100 mm.[19] Based on the above, the Dutch climate is unsuited for cotton growth.

Climate conditions in other parts of the European Union e.g. Greece, Spain, Bulgaria and Portugal are more suitable for growing cotton.[9] Seeds from cotton cultivars do not possess dormancy[7,8] and will germinate in autumn if conditions are favourable. In addition, seeds will usually not survive in humid soil.[8] In regions with mild and dry winters, cottonseeds may overwinter and germinate in spring.[6] The occurrence of volunteer cotton is limited by the availability of adequate soil moisture or the occurrence of frost.[8]

There is no indication that the introduced traits, which confer resistance to certain lepidopteran pests and tolerance to glufosinate ammonium containing herbicides, will increase the ability of cotton to survive in the environment. The applicant carried out an agronomic assessment for GHB119. The COGEM is of the opinion that the agronomic assessment does not give any indication to assume that GHB119 has an increased survivability compared to conventional cotton lines. Furthermore it is unlikely that cotton will grow in the Netherlands.

In view of the above, there are no reasons to assume that GHB119 has an increased potential for the establishment of feral populations in case of incidental spillage because the climate in North West Europe is not suited for cotton growth.

Since 2008 COGEM abstains from giving advice on the potential risks of incidental consumption in case a food/feed assessment is already carried out by other organisations.[20] This application is submitted under Regulation (EC) 1829/2003, therefore a food/feed assessment is carried out by EFSA. Other organisations who advise the competent authorities can perform an additional assessment on food safety although this is not obligatory. In the Netherlands a food and/or feed assessment for Regulation (EC) 1829/2003 applications is carried out by RIKILT. Regarding the risks for food and feed, the outcome of the assessment by other organisations (EFSA, RIKILT) was not known at the moment of the completion of this advice.

General surveillance plan

General surveillance (GS) has been introduced to be able to observe unexpected adverse effects of genetically modified (GM) crops on the environment. The setting or population in which these effects might occur is either not, or hardly predictable. The GS plan states that unanticipated adverse effects will be monitored by existing monitoring systems which include the authorisation holder and operators involved in the handling and use of viable cotton line GHB119. In 2010, COGEM formulated criteria which GS plans concerning Dutch applications for import and cultivation of GM crops have to comply with.[21] COGEM concluded that the GS plans could be improved by a guarantee that operators will monitor for unanticipated effects. In the present GS plan on cotton line GHB119 the authorization holder states that the operators have agreed to provide information relevant to the monitoring of GHB119 to the authorisation holder. More important, it is stated that the authorisation holder will be able to give evidence that the operators collect this information. This is in line with the criteria laid down by COGEM.[21]

In the EFSA guidance document, EFSA states that raw data and analysis of monitoring data should be made available by the applicant to the Competent Authorities and the European Commission.[22] COGEM agrees with this request and points out that the General Surveillance plan of GHB119 cotton could be improved by a statement of the applicant on this point.[23]

Advice

The present application concerns import and processing for feed and food purposes of the genetically modified cotton line GHB119. Cultivation is not part of the application. Therefore, the risk assessment focuses on the accidental spillage of cottonseeds.

Cotton plants are very sensitive to temperature. The North Western European climate is unsuited for cotton growth. There is no indication that the introduced traits, which confer resistance to certain lepidopteran pests and tolerance to glufosinate ammonium containing herbicides, will increase the ability of cotton to survive in the environment. Therefore, there are no reasons to assume that GHB119 has an increased potential for the establishment of feral populations in case of incidental spillage. The climate in North West Europe is not suited for cotton growth. In COGEM's view there is no risk that incidental spillage of cottonseeds will lead to the spread of cotton within North West Europe. The molecular characterisation of GHB119 meets the criteria of COGEM.

In view of the above, COGEM is of the opinion that the risks for humans and the environment associated with import and processing of cotton line GHB119 are negligible. A food/feed safety assessment is carried out by other organisations. Therefore, COGEM abstains from advice on the potential risks of incidental consumption.

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