Import and processing of cotton MON88913 x MON15985

COGEM advice CGM/080328-01

Summary

COGEM has been asked to advice on an application concerning the import and processing for use in food and feed of a hybrid cotton line. Cultivation is not part of this application.

The cotton line MON88913 x MON15985 was produced by cross-breeding two genetically modified cotton lines. The hybrid line contains two cp4 epsps genes and the cry1Ac and cry2Ab2 genes. As a result, this line is tolerant to glyphosate-containing herbicides and is resistant to insects of the Lepidopteran order. The cotton line is grown commercially in Australia and South Africa.

In Europe, no wild relatives of cotton are present and modern cotton cultivars do not possess any of the attributes commonly associated with problematic weeds. There are no reasons to assume that the genes inserted will increase the potential of the cotton to establish feral populations. Furthermore, establishment of feral populations in cotton producing countries is never observed. Moreover, cotton cannot survive the climatological conditions in Northwest Europe. COGEM is of the opinion that without irrigation, cotton volunteers cannot survive and establish themselves in the wild. Therefore, COGEM is of the opinion that incidental spillage of seeds of this cotton line will not pose a risk to the environment in the Netherlands nor in Northwest Europe.

Furthermore, COGEM is of the opinion that the molecular characterisation is performed adequately.

In view of these considerations, COGEM is of the opinion that the import and processing of cotton line MON88913 x MON15985 does not pose a significant risk to the environment in the Netherlands.

Introduction

The present application by Monsanto Europe S.A., file EFSA/GMO/UK/2007/42, concerns the import and processing of the hybrid cotton line MON88913 x MON15985 for use in food and feed. Cotton is mainly cultivated for the use of cotton lint. Cottonseeds are harvested as rest products and used as feed, or for the production of cottonseed oil for human consumption.

MON88913 x MON15985 expresses two *cp4 epsps* genes which confer tolerance to glyphosate-containing herbicides. Furthermore, the hybrid line expresses the *cry1Ac* and *cry2Ab2* genes, both of which confer resistance to Lepidopteran pests.

In Australia and South Africa, the cotton line MON88913 x MON15985 has been authorized for commercial import, processing and cultivation in 2006 and 2007 respectively (1). Parental line MON88913 has been approved for commercial import, processing and cultivation in the United States of America (2004/2005), Australia (2006) and South Africa (2007). Furthermore, it has been approved for food and feed purposes in Japan (2005/2006), Korea (2006), Mexico (2006) and the Philippines (2005) (1). There is a history of safe use e.g. no adverse health effects concerning handling and consuming of products and derivatives of this line have been reported.

Aspects of the crop

Cotton is a member of the genus *Gossypium* and belongs to the *Malvacea* family (2). More than 95% of commercial cotton is upland cotton, *G. hirsutum*, while long staple cotton, *G. barbadense,* occupies a small area of less then 5% (3).

Major producers of seed cotton and lint are China, the United States of America, India, Pakistan, Brazil and Turkey. Together, these countries are responsible for 80% of the total cotton production (4). Within the European Union, cotton is mainly grown in Greece and on a smaller scale in Spain and Bulgaria (5). It should be noted that only non-genetically modified (gm) cotton is grown in Europe.

Depending on cultivar and climate, the growth period can range from 160 to 220 days. The crop will flower about eight weeks after planting. In the following two months, a cottonboll will develop and will finally open. About eight weeks later, the cotton fibers have reached full length and cellulose content and the cotton can be harvested (6).

Cotton is highly sensitive to temperature. It does not start its vegetative activity until the temperature reaches 15ºC and the activity is delayed when the temperature rises above 38ºC. 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 the vegetative branches increases and the cropping period will be extended. Reduced light intensity will retard flowering and fruiting. Because cotton is susceptible to frost, the whole growth period of six months has to be free of frost (3,7).

In areas where the rainfall is less then 500 mm a year, irrigation should be applied (8). In places where cotton is grown as a rain-fed crop, the average rainfall is 800-1200 mm (6).

Cultivated cotton is predominantly a self-pollinating species. But the prevalence of insects strongly influences outcrossing rates for cotton. Many field-based assessments estimate out-crossing rates at 10% or less, although rates up to 80% have been found. The pollen remains viable up to a period of twelve hours (8).

Cotton has some wild-relatives, however, they are not found in Europe (3).

Cotton is a domesticated crop. 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 (8). Cotton volunteers occur in cotton growing areas and are relatively common where cotton seed is used as lifestock feed. There is no indication, however, that these volunteers establish feral populations. Seeds that do not germinate are likely to be removed by seed predators or rot, rather than become incorporated into a persistent soil seed bank (8).

Previous COGEM advices

Recently, COGEM advised positively on the import and processing of gm parental cotton line MON88913 (9). In 1998, COGEM also advised positively on the commercialisation of cotton line MON531 (10). MON531 was used to produce the parental line MON15985.

In the past, COGEM has given a positive advice on a gm cotton line containing two *cry* genes (insect resistance) in combination with a *pat* gene (herbicide tolerance) for import and processing (2,11).

Molecular characterisation

The hybrid cotton line MON88913 x MON15985 was produced by cross-breeding the two gm parental cotton lines MON88913 and MON15985. An overview of the introduced sequences in the parental lines is given below:

<u>Summary of the elements inserted in MON88913</u>

- *cp4 epsps* gene cassette 1
  - P-*FMV/Tsf1*, promoter derived from the *Figwort mosaic virus*/*Arabidopsis thaliana*
  - L-T*sf1*, leader sequence from *A. thaliana*
  - I-T*sf1,* intron sequence from *A. thaliana*
  - TS-*ctp2*, targeting sequence from *A. thaliana*, directs CP4 EPSPS to the chloroplasts
  - CS-*cp4 epsps,* gene from *Agrobacterium tumefaciens*, confers tolerance to glyphosate containing herbicides
  - T-*E9*, terminator derived from *Pisum sativum*, ends transcription.

- *cp4 epsps* gene cassette 2
  - P-*35S/act8*, promoter derived from the *Cauliflower mosaic virus / A. thaliana*
  - L-*act8,* leader sequence from *A. thaliana*
  - I-*act8*, intron sequence from *A. thaliana*
  - TS-*ctp2*, targeting sequence from *A. thaliana*, directs CP4 EPSPS to the

chloroplasts

- CS-*cp4 epsps*, gene from *A. tumefaciens*, confers tolerance to glyphosate containing herbicides
- T-*E9*, terminator derived from *P. sativum*, ends transcription.

Summary of the elements inserted in MON15985

MON15985 was produced by inserting the coding sequence for CRY2Ab2 into the genome of the genetically modified cotton line MON531, which already contained the *cry1Ac* gene.

- Elements associated to the functional copy of the *cry1Ac* insert (MON531):
  - T-*7S*, terminator derived from soybean 7S, ends transcription
  - CS-*cry1Ac*, gene from *Bacillus thuringiensis*, confers resistance to insects of the *Lepidopteran* order
  - P-*e35S*, promoter originating from the *Cauliflower mosaic virus* (CaMv), containing the duplicated enhancer region
  - *aad*, (non functional) bacterial gene coding for an aminoglycoside-modifying enzyme from transposon Tn7
  - CS-*nptII*, coding sequence for neomycin phosphotransferase type II from transposon Tn5.

- Elements associated to the *cry2Ab2* insert:
  - P-*e35S*, promoter originating from CaMv, containing the duplicated enhancer region
  - L-*Hsp70*, leader sequence from petunia heat shock protein
  - TS-*ctp2*, targeting sequence from *A. thaliana*, directs Cry2Ab2 to the cholorplasts
  - CS-*cry2Ab2*, gene from *B. thuringiensis*, confers resistance to insects of the *Lepidopteran* order
  - T-*nos*, terminator from *A. tumefaciens*, ends transcription.

Properties of the introduced genes conferring herbicide tolerance

The cotton line was genetically modified with two *cp4 epsps* genes encoding the protein CP4 EPSPS possessing a high tolerance to glyphosate. EPSPS is a naturally occurring enzyme involved in the biosynthesis of aromatic amino acids. In non transgenic cotton lines, glyphosate acts by binding to and inhibiting the function of naturally occurring EPSPS. Consequently, aromatic amino acids are no longer formed, leading to plant death. In contrast, CP4 EPSPS is not affected by glyphosate because of a reduced binding affinity. Because MON88913 x MON15985 expresses *cp4 epsps*, it has acquired a high tolerance to glyphosate (12).

EPSPS proteins are active in the chloroplasts of a plant cell. The sequence encoding the chloroplast transit peptide is fused to the *cp4 epsps* gene, resulting in the transport of the transgenic CP4 EPSPS protein to the chloroplast (13).

Properties of the introduced genes conferring insect resistance

Cotton line MON88913 x MON15985 also contains genes encoding the proteins Cry1Ac en Cry2Ab2. These δ-endotoxins provide increased resistance to certain insects of the *Lepidopteran* order, among others the cotton bollworm (*Helicoverpa armigera*) and pink bollworm (*Pectinophora gossypiella*). Larvae of these insects inflict damage to the plants through feeding.

The δ-endotoxins confer protection by solubilizing in the midgut of susceptible insects, after which activation by midgut proteases takes place to release a toxin fragment. The toxin fragment binds to specific receptors on the epithelial surface of the midgut. Subsequently, pores are formed in the membranes of the gut cells of the insect, enabling midgut bacteria to enter the body cavity, which leads to septicemia and death (14).

Molecular analysis

The applicant has supplied studies on the molecular analysis of the two parental cotton lines MON88913 and MON15985. Southern blot and sequencing showed that intact copies of the inserts are present in the parental lines. Furthermore, the inserts and the cotton genomic DNA flanking the 3'and 5'end of the inserts were sequenced. Moreover, no vector backbone sequences are present in both lines.

In COGEM's opinion, the molecular analysis of both parental cotton lines was performed adequately and completely. Therefore, it is sufficient that the applicant has proven by Southern blot that the inserts are presents in the hybrid cotton line.

General surveillance plan

A general surveillance plan is supplied by the applicant. COGEM is of the opinion that the applicant should describe in more detail how the general surveillance will be organized and should indicate which organizations will be involved. However, in the opinion of COGEM the surveillance plan is adequate for the Netherlands, because cotton cannot survive the Northwestern European climate. In the specific case of the Netherlands, a more detailed general surveillance plan is therefore of less importance.

Advice

The present application concerns the import and processing for feed and food purposes of a gm cotton line. Cultivation is not part of the application. Therefore, the risk assessment focuses on the accidental spillage of cottonseeds. As stated above, cotton plants are very sensitive to temperature. 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 Dutch climate is far from ideal for growing cotton. During the warmest months (April to October), the average temperature is around 14ºC. The average rainfall for spring and summer is 375 mm and is below the required 500 mm (15). Moreover, the frost periods during the winter make it impossible for cotton to survive and establish itself in the Netherlands.

Climate conditions in other parts of the European Union are more suitable for growing cotton. At the moment cotton is grown in Greece, Spain and Bulgaria (5). However, it is not to be expected that the spillage of cottonseeds in these countries leads to the establishment of feral populations because no self sustaining feral populations have been observed in the world. All European cotton is irrigated due to shortage of rainfall in the growing season. Furthermore as stated above, modern cotton cultivars do not possess any of the attributes commonly associated with problematic weeds and there are no reasons to assume that the inserted genes will increase the potential of the cotton to run wild.

In view of the above, COGEM is of the opinion that there is no risk that incidental spillage of cottonseeds will lead to the spread of cotton within Northwest Europe. Therefore, COGEM is of the opinion that the proposed import and processing of cotton line MON88913 x MON15985 does not pose a significant risk for the environment in the Netherlands.

Additional remarks

Cotton line MON88913 x MON15985 contains the antibiotic resistance genes *nptII* (which confers resistance to kanamycin and neomycin) and *aad* (conferring resistance to streptomycin and spectinomycin) (16). In 2004, the European Food Safety Authority (EFSA) published an opinion on the use of antibiotic resistance genes as marker genes in genetically modified plants. They conclude that the frequency of horizontal gene transfer of antibiotic resistance genes from genetically modified plants to other organisms is very low. Furthermore, they state that it has been shown, or is extremely likely, that there is a considerable pool of resistance genes already present in the microbiota in the environment. In spite of these considerations, EFSA is of the opinion that the *aad* gene should be restricted to field trial purposes and should not be present in gm plants which will be placed on the market (17).

On the other hand, EFSA states that there is no rationale for restricting or prohibiting the use of *nptII* in plants to be placed on the market; in particular, because *nptII*, among others, has a history of safe use in food crops (17).

COGEM points out that the chance of gene transfer from plant to bacterium is not likely to occur and has only been observed during specific laboratory situations and not in practice (16;18). Furthermore antibiotic resistance genes are already present in the environment (16).

It is unclear whether the EFSA opinion concerns cultivation or cultivation and import. In cultivation plant material is in close contact with soil bacteria; however such contact does not take place in case of import. Moreover, consumption of cotton products is limited.

In view of the above, COGEM has already approved the commercialisation of MON531 (used for the production of parental cotton line MON15985), which contains the *nptII* and *aad* genes (10). Furthermore, MON531 is approved for food and feed purposes in the European Union. In COGEM's opinion, the presence of both genes poses no risk to the environment.

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