

Aan de minister van
Volkshuisvesting, Ruimtelijke
Ordening en Milieubeheer
Mevrouw dr. J.M. Cramer
Postbus 30945
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DATUM: 18 december 2007
KENMERK: CGM/071218-01
ONDERWERP: Advies import en verwerking van herbicidentolerante katoen MON88913
(EFSA/GMO/UK/2007/41)

Geachte mevrouw Cramer,

Naar aanleiding van de adviesvraag betreffende het dossier EFSA/GMO/UK/2007/41, getiteld "Application for authorization of MON 88913 cotton in the European Union, according to Regulation (EC) No 1829/2003" door Monsanto Europe S.A., adviseert de COGEM als volgt.

Samenvatting:

De COGEM is gevraagd te adviseren over import van genetisch gemodificeerde katoen. Onderhavige katoenlijn is voorzien van twee *cp4 epsps* genen waardoor de planten tolerant zijn geworden voor toediening van herbiciden met als werkzame stof glyfosaat.

In Europa komen geen wilde verwanten van katoen voor en katoen bezit niet de eigenschappen om te kunnen verwilderen. Katoen is sterk koudegevoelig, heeft hoge temperaturen nodig voor kieming en ontwikkeling van de plant. Daarbij is irrigatie of hoge neerslag gedurende het hele jaar noodzakelijk voor de teelt van katoen. Er zijn geen redenen om aan te nemen dat de modificatie het verwilderingspotentieel vergroot. De COGEM acht derhalve de kans verwaarloosbaar klein dat incidenteel morsen van de katoenzaden leidt tot verspreiding van deze genetisch gemodificeerde katoen binnen Europa. Daarnaast heeft de moleculaire karakterisering van de lijn aangetoond dat de risico's van incidentele consumptie van deze katoenlijn verwaarloosbaar klein zijn.

Concluderend heeft de COGEM geen bezwaar tegen import en verwerking van onderhavige katoenlijn en worden de risico's voor mens en milieu verwaarloosbaar klein geacht.

De door de COGEM gehanteerde overwegingen en het hieruit voortvloeiende advies treft u hierbij aan als bijlage.

Hoogachtend,

A handwritten signature in black ink, consisting of a large, stylized loop on the left and a long, horizontal stroke extending to the right.

Prof. dr. ir. Bastiaan C.J. Zoeteman

Voorzitter COGEM

c.c. Dr. ir. D.C.M. Glandorf

Dr. I. van der Leij

Import and processing of herbicide tolerant cotton MON88913

COGEM advice CGM/071218-01

Summary

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

The recombinant cotton line MON88913 is genetically modified by insertion of two cp4 epsps genes. As a result, MON88913 confers tolerance to herbicides containing the active ingredient glyphosate. The cotton line is already commercially grown in the United States of America, 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 run wild. Furthermore, establishment of feral populations in cotton producing countries is never observed. Cotton can not survive the climatological conditions in North-West Europe. COGEM is of the opinion that without irrigation, cotton volunteers can not survive and establish themselves in the wild. Therefore, COGEM is of the opinion that incidental spillage of seeds of this cotton line will pose no risk to the environment in the Netherlands nor in Europe.

Furthermore, COGEM is of the opinion that the molecular characterisation is performed adequately. Based on the molecular characterisation COGEM is of the opinion that there are no reasons to assume that the incidental consumption of the cotton line will pose a health risk.

In view of these considerations, COGEM is of the opinion that the proposed import and processing of cotton line MON88913 does not pose a significant risk for human health and the environment in the Netherlands.

Introduction

The present application by Monsanto Europe S.A., file EFSA/GMO/UK/2007/41, concerns the import and processing of cotton line MON88913 for use in feed and food. Cotton is mainly cultivated for the use of cotton lint. Cottonseeds are harvested as rest products and used for feeding purposes, or for the production of cottonseed oil for human consumption.

The cotton line contains and expresses two cp4 epsps genes which confer tolerance to herbicides containing the active ingredient glyphosate.

MON88913 has been previously approved for commercial import, processing and cultivation in the United States of America, South Africa and Australia. Furthermore,

it has been approved for food and feed purposes in Canada, Japan, Korea, Mexico and the Philippines (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.

Previous COGEM advices

In the past COGEM has advised positively on the genetically modified cotton line containing 2 *cry* genes (insect resistance) in combination with a *pat* gene (herbicide tolerance) for import and processing (2,3). Besides, the COGEM advised positively on cotton line LLConton25 which contains the *bar* gene conferring resistance to glufosinate ammonium based herbicides.

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 than 5% (5).

The major producers of seed cotton and lint are China, 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 grown on a small scale in Greece and Spain (9). It should be noted that only non-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. The time taken for the cotton fibers to reach full length and cellulose content, is about eight weeks later. After this, the cotton can be harvested (10).

Cotton is highly sensitive to temperature. It does not start its vegetative activity until the temperature reaches 15°C and the activity is delayed when temperature rises above 38°C. For normal development, cotton needs an average of 150 days with temperatures between these values (7). The optimum temperature for germination is 34°C, for the growth of seedlings 24-29°C and for later continuous growth 34°C. 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 (5,7).

In areas where the rainfall is less than 500 mm a year, irrigation should be applied (6). In places where cotton is grown as a rain-fed crop, the average rainfall is 800-1200 mm (10).

Cultivated cotton is 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 (6).

Cotton has some wild-relatives, however, they are not found in Europe. Besides, many wild relatives are diploid ($2n = 26$) making them incompatible with the cultivated species *G. hirsutum* and *G. barbadense* which are tetraploid ($4n = 52$) (5).

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 (6). Cotton volunteers occur in cotton growing areas and are relatively common where cotton seed is used as livestock 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 (6).

General surveillance plan

A general surveillance plan is supplied by the applicant. As stated in previous advices, 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 are involved. In addition, the applicant should ascertain that information on eventual adverse effects is indeed obtained. Furthermore, direct and indirect effects should be reported annually.

However, because cotton can not survive in the North-Western European climate, a general surveillance plan is in this specific case of less importance for the situation in the Netherlands.

Molecular characterisation

Cotton line MON88913 is genetically modified by means of *Agrobacterium tumefaciens* transformation. Two tandem *cp4 epsps* expression cassettes were transferred into the cotton genome.

cp4 epsps gene cassette 1

- P-FMV/*Tsf1*, promoter derived from the *Figwort mosaic virus/Arabidopsis thaliana*
- L-*Tsf1*, leader sequence from *A.thaliana*
- I-*Tsf1*, intron sequence from *A.thaliana*
- TS-*ctp2*, targeting sequence from *A.thaliana*, directs CP4 EPSPS to the chloroplasts
- CS-*cp4 epsps*, gene confers resistance 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 confers resistance to glyphosate containing herbicides
- T-*E9*, terminator derived from *P. sativum*, 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 expresses *cp4 epsps*, it has acquired a high tolerance to glyphosate (11).

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 (12).

Molecular analysis

In COGEM's opinion, the applicant has proven by Southern blot that a single intact copy of the insert is present in 356043. No vector backbone sequences were present in the transgenic line. Results obtained by PCR amplification and DNA sequence analysis confirm that the flanking regions of the insert consist of genomic cotton DNA.

The junction between the T-DNA and its flanking regions was examined for the presence of potential novel open reading frames (ORFs). Sequences spanning the 5'cotton genomic DNA-inserted DNA junction and the 3'inserted DNA-cotton genomic DNA junction were translated from stop codon to stop codon in all six reading frames. Subsequently, putative polypeptides were compared to both a dataset of known and putative allergens and to publicly available protein datasets. Results from the comparisons showed that the peptide did not match to known or putative allergens or toxins.

In conclusion, COGEM is of the opinion that the molecular characterisation was adequately performed. Based on the molecular characterisation COGEM is of the opinion that there are no reasons to assume that the incidental consumption of the cotton line will pose a health risk.

Advice

The present application concerns the import and processing for feed and food purposes of a cotton line. Cultivation of the present line is not part of the application. The risk assessment therefore focuses on the accidental spillage of cottonseeds. As stated above, cotton growth is very sensitive to temperature. A reasonably high temperature 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 14°C. The average rainfall for spring and summer is 375 mm and is below the required 500 mm (8). Moreover, the fact that the Netherlands are known for their frost periods in winter makes it impossible for cotton to survive and to 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 and Spain (9). However, it is not to be expected that the spillage of cottonseeds can lead to the establishment of feral populations because cotton has never ever established self sustaining feral population anywhere in the world. All European cotton is irrigated due to the lack 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. Furthermore, establishment of feral populations in cotton producing European countries is never observed.

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 the European Union. Besides, COGEM is of the opinion that the applicant did prove that the risks of incidental consumption of cottonseeds are negligible. Therefore, COGEM is of the opinion that the proposed import and processing of cotton line MON88913 does not pose a significant risk for human health and the environment in the Netherlands.

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