# Import and processing of genetically modified herbicide tolerant cotton MON88701

## COGEM advisory report CGM/130919-02

**Summary**

*The present application by Monsanto Europe S.A. concerns import and processing for use in feed and food of the genetically modified cotton event MON88701 (EFSA/GMO/NL/2013/114). Cultivation is not part of this application.*

*Cotton line MON88701 was produced by* Rhizobium radiobacter *mediated transformation of conventional cotton. MON88701 expresses the* dmo *and* bar *genes. As a result, MON88701 cotton is tolerant to dicamba and glufosinate ammonium containing herbicides.*

*In Northwestern Europe, no wild relatives of cotton are present. Modern cotton cultivars do not possess any of the attributes commonly associated with problematic weeds. The introduced traits will not introduce a potential to establish feral populations. Moreover, cotton cannot survive in Northwestern Europe due to its climate. Therefore, COGEM is of the opinion that incidental spillage of MON88701 seeds will not pose a risk to the environment in Northwestern Europe.*

*In view of the above, COGEM is of the opinion that import and processing of cotton line MON88701 poses a negligible risk to man and the environment. 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 application by Monsanto Europe S.A. (EFSA/GMO/NL/2013/114) concerns import and processing of the genetically modified cotton event MON88701. This cotton event was produced by *Rhizobium radiobacter* (previously known as *Agrobacterium tumefaciens*)[1] mediated transformation of *Gossypium hirsutum* and expresses the *dmo* and *bar* genes. As a result, MON88701 is tolerant to dicamba and glufosinate ammonium containing herbicides. The application also includes conventional breeding products of event MON88701 with other *Gossypium* species.

**Previous COGEM advisory reports**

MON88701 has not been previously assessed by COGEM. However, in the past COGEM has advised positively on import of several cotton events expressing the *bar* gene.[2,3,4] Furthermore, in 2011, COGEM advised positively on import and processing of genetically modified soybean MON87708 expressing the *dmo* 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 is *Gossypium hirsutum* (90%) and *Gossypium barbadense* (5%), while *Gossypium*

*arboreum* and *Gossypium herbaceum* are cultivated as well.[6,7,8] The only species cultivated cotton in Europe is *G. hirsutum*, that is grown in Greece, Spain and Bulgaria.[9]

Cotton plants reproduce sexually.[8] Cotton is predominantly a self-pollinating species, but cross-pollination may occur. Dissemination of pollen by wind is (almost) absent.[7,8] The pollen of cotton is large, heavy and somewhat sticky.[7,8] Outcrossing rates for cotton are strongly influenced by the presence of insects. Pollinators of cotton flowers include bumblebees (*Bombus* spp.), honeybees (*Apis* spp.) and other bee species (*Anthophora* spp., *Melissodes* spp. and *Halictus* spp.).[7,10] Hybridisation between *G. hirsutum* and *G. barbadense* may occur and can lead to viable progeny (F1). Hybrid F2 progeny contain either depauperate types or plants that closely resemble one of the parents.[10] Wild relatives of cotton (*Gossypium* spp.) do not occur in Northwestern Europe. Therefore, hybridisation with wild relatives cannot occur in Northwestern Europe.[7]

Cotton is highly sensitive to temperature and susceptible to frost. Temperature is the main factor which determines the geographic range in which cotton can be grown. Plant development ceases below a temperature of 12 ºC and delays when the temperature rises above 38 ºC.[7,8] *G. hirsutum* needs a period of 180 to 200 frost-free days for normal maturation, with an average of 150 days of suitable temperatures (averaging 21-22 ºC). The optimal temperature for growth is between 30 and 35 ºC.[11] However, *G. barbadense* has a longer growing season and needs 200-250 frost-free days and a lower optimum temperature for growth (25-30°C).[7,12] In places where cotton is cultivated 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 is necessary.[7]

Cottonseed can be dispersed by wind, water, during transport or when feeding cattle.[8] In addition, cottonseed can be transported by birds or rodents. Seeds from cotton cultivars do not possess dormancy and will germinate directly if conditions are favourable.[7,8] Seeds usually do 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 seed 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 presence of volunteer cotton is limited by soil moisture content and frost.[8] There are reports that *G. hirsutum* and *G. herbaceum* cotton are naturalised in some Southern European countries, e.g. Greece and Spain.[13,14]

**Molecular characterization**

Cotton MON88701 was developed by *R. radiobacter* mediated transformation of tissue from the *G. hirsutum* cotton variety Coker 130 using vector PV-GHHT6997 containing a T-DNA region with two expression cassettes. Dicamba and glufosinate were used as the selective agents.

The following elements were introduced in MON88701:

- Intervening sequence. Sequence used in DNA cloning.

- *PC1SV* promoter. Promoter from the Full-Length Transcript of *Peanut chlorotic streak virus* that directs transcription in plant cells.
- Intervening sequence. Sequence used in DNA cloning.
- *TEV* leader. 5'non-translated region from the *Tobacco etch virus* genome that is involved in regulating gene expression.
- Intervening sequence. Sequence used in DNA cloning.
- *CTP2* targeting sequence. Targeting sequence of the *ShkG* gene from *Arabidopsis thaliana* encoding the EPSPS transit peptide region that directs transport of the protein to the chloroplast.
- Modified *dmo* gene. Codon optimized coding sequence of the dicamba mono-oxygenose gene from *Stenotrophomonas maltophilia* that confers dicamba tolerance.
- Intervening sequence. Sequence used in DNA cloning.
- *E6* terminator. 3' Untranslated region sequence of the *E6* gene from *G. barbadense* that directs polyadenylation of mRNA.
- Intervening sequence. Sequence used in DNA cloning.
- *e35S* promoter. Promoter from the 35S RNA of *Cauliflower mosaic virus* containing the duplicated enhancer region that directs transcription in plant cells.
- Intervening sequence. Sequence used in DNA cloning.
- *Hsp70* leader. 5'non-translated region of the *dnaK* gene from *Petunia hybrida* (that encodes heat shock protein 70) that regulates gene expression.
- Intervening sequence. Sequence used in DNA cloning.
- *bar* coding sequence. Coding sequence for the phospinthricin N-acetyltransferase (PAT) protein of *Streptomyces hygroscopicus* that confers glufosinate ammonium tolerance.
- Intervening sequence. Sequence used in DNA cloning.
- *nos* terminator. 3'Untranslated region sequence of the nopaline synthase (*nos)* gene from *R. radiobacter* that directs polyadenylation of mRNA.
- Intervening sequence. Sequence used in DNA cloning.
- Left border. 442 bp sequence from *R. radiobacter* containing the Left Border sequence used for transfer of the T-DNA.

***Expressed proteins conferring herbicide tolerance***

The *dmo* gene from the bacteria *S. maltophilia* encodes dicamba mono-oxygenase (DMO). The properties of low doses of dicamba resemble those of natural plant hormones (auxins) involved in plant development processes. High concentrations of dicamba in plant tissues induce abnormal and uncontrollable growth and disruption of normal plant functions, resulting in death. DMO demethylates dicamba to the non-herbicidal compounds 3,6-dichlorosalicylic acid and formaldehyde, rendering it inactive.

The *dmo* gene introduced in MON88701 is codon optimized for optimal expression in cotton, conferring tolerance to dicamba. Compared to the wild-type DMO the MON88701 DMO contains an insertion of a leucine at position 2, and an additional 9 amino acids at the N-terminus that remain after cleavage of CTP2.

MON88701 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.[15] MON88701 expresses the *bar* gene with native codon usage derived from *S. hygroscopicus* 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.[15] As a result MON88701 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 MON88701 cotton contains one single copy of the T-DNA. The applicant further showed that MON88701 does not contain backbone sequences of the vector plasmid PV-GHHT6997.

Bioinformatic analysis showed that the 5' and 3' flanking sequences of the integrated DNA are cotton genomic DNA. A comparison with the parental sequence revealed a deletion of 123 bp of the cotton genomic DNA at the site of insertion in MON88701.

The junctions between the T-DNA insert and the flanking cotton genomic DNA, and the junctions between the elements of the T-DNA insert were screened for potential newly created open reading frames (ORFs). The ORFs were defined as sequences between two stop codons with a coding capacity of minimal eight amino acids. In the 5' and 3' junction regions nine putative ORFs were identified. Furthermore, the six reading frames of the inserted T-DNA were translated *in silico* and eight putative proteins were studied further. Among these proteins were DMO and PAT. The other putative proteins were located in the reverse complement strands of the 35S RNA promoter or the *bar* coding sequence. The applicant compared the deduced amino acid sequences of the putative proteins from the junctions and the insert with known toxins, allergens and proteins (TOX, AD and PRT databases, 2012). Bio-informatic analyses showed no biologically significant sequence similarities with known proteins, toxins and allergens.

In view of the above, COGEM is of the opinion that the molecular characterisation of MON88701 has been adequately performed and meets the criteria laid down by COGEM.[16]

**Environmental risk assessment**

Cotton is predominantly a self-pollinating species, but cross-pollination may occur. Wild relatives of cotton (*G. hirsutum and G. barbadense*) are not present in Northwestern Europe and therefore, hybridisation with wild relatives is excluded.[7]

Cotton plants are susceptible to frost. A reasonably high temperature (optimally 25-30 ºC for *G. barbadense* and 30-35 ºC for *G. hirsutum*) is required in all stages of development. For normal maturation, a period of 200 to 250 frost-free days is needed for *G. barbadense* and 180 to 200 days for *G. hirsutum*. In addition, this period needs to have on average 150 days of suitable temperatures, averaging 21-22 ºC. In the Netherlands, the summer months of May, June, July, August and September (in total 153 days) have average monthly temperatures above 12 ºC, but below 18 ºC.[17] In addition, in areas where rainfall is less than 500 mm a year, irrigation should be

applied for cotton growth. In the months of May through September in the Netherlands, the average monthly precipitation does not exceed 100 mm.[17] Based on the above, the current Dutch climate is unsuited for cotton growth.

There is no indication that the introduced traits, which confer tolerance to dicamba and glyphosate containing herbicides, will introduce an ability of cotton to survive in the environment. The applicant carried out an agronomic assessment for MON88701. The agronomic assessment does not give any indication to assume that MON88701 has an increased fitness compared to conventional varieties.

In view of the above, there are no reasons to assume that MON88701 has an increased potential for the establishment of feral populations in case of incidental spillage of cottonseed because the climate in Northwestern 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.[18] 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 applicant's general surveillance plan is in line with the criteria COGEM formulated in 2010.[19]

COGEM notes that the GS plan of MON88701 could be improved by a statement that raw data and analysis of monitoring data will be made available to the Competent Authorities and the European Commission.[20] The same remark has been made by EFSA in its guidance document.[21]

**Advice**

The present application concerns import and processing for feed and food purposes of the genetically modified cotton line MON88701. This application includes import and processing of all *Gossypium* species (specifically *G. hirsutum* and *G. barbadense*) derived from MON88701 by conventional breeding methods.

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 Northwestern European climate is unsuited for cotton growth. There is no indication that the introduced traits, which confer tolerance to dicamba and glufosinate ammonium containing herbicides, will introduce an ability to survive in the environment. Therefore, incidental spillage of cottonseeds will not lead to feral cotton populations in Northwestern Europe. COGEM considers the current GS plan sufficient for import

and processing of cotton line MON88701. The molecular characterisation of MON88701 meets the criteria of COGEM.

In view of the above, COGEM is of the opinion that the risks for man and the environment associated with import and processing of cotton line MON88701 are negligible. A food/feed safety assessment is carried out by other organisations. Therefore, COGEM abstains from advice on potential risks of incidental consumption.

**References**


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