PART III

INFORMATION REQUIRED BY THE CARTAGENA PROTOCOL ON BIOSAFETY, UNDER THE CONVENTION ON BIOLOGICAL DIVERSITY

The information provided in this part (Part III) complies with Annex II to Cartagena Protocol and can be notified to the Biosafety Clearing-House by the European Commission as provided for in Article 44 of Regulation (EC) No 1829/2003.

A. Name and contact details of the applicant for a decision for domestic use.

This is an application submitted by Bayer CropScience AG.

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B. Name and contact details of the authority responsible for the decision

European Commission
Rue de la Loi/Wetstraat 200
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C. Name and identity of the LMO

GHB614xLLCotton25xMON 15985 cotton has been obtained by conventional crossing of lines containing the single events: GHB614, LLCotton25 and MON 15985. No new genetic modification was used for the development of GHB614xLLCotton25xMON 15985 cotton.

The unique identifier assigned to GHB614xLLCotton25xMON 15985 cotton is:
BCS-GHØØ2-5 x ACS-GHØØ1-3 x MON-15985-7

D. Description of the gene modification, the technique used, and the resulting characteristics of the LMO

GHB614xLLCotton25xMON 15985 cotton has been obtained by conventional crossing of lines containing the single events: GHB614, LLCotton25 and MON 15985. No new genetic modification was used for the development of GHB614xLLCotton25xMON 15985 cotton.

E. Any unique identification of the LMO

The unique identifier assigned to GHB614xLLCotton25xMON 15985 cotton is:
BCS-GHØØ2-5 x ACS-GHØØ1-3 x MON-15985-7

F. Taxonomic status, common name, point of collection or acquisition, and characteristics of recipient organism and/or parental organisms related to biosafety

(a) Family name:	Malvaceae
(b) Genus:	Gossypium
(c) Species:	hirsutum
(d) Subspecies:	Not applicable
(e) Cultivar/breeding line:	GHB614, LLCotton25, MON 15985
(f) Common name:	cotton
Point of collection:	commercial variety of USA and other countries
Characteristics related to biosafety:	not different from other cotton varieties except for tolerance to herbicides glyphosate and glufosinate ammonium and to resistance to certain pests of the Lepidoptera family.

G. Centres of origin and centres of genetic diversity, if known, of the recipient organism and/or parental organisms and a description of the habitats where the organisms may persist or proliferate

Plants of the tribe *Gossypiae* originated in the tropics and subtropics. Except as a cultivated crop, they are essentially excluded from temperate climates. They also tend to be plants of the southern hemisphere. Geographical distribution for the cotton crop is located between 42° Latitude N. (Central Asia, China) and 30° Latitude S. (Australia, Northern Argentina). Thus, cotton is a plant of tropical origin, but presently more than 50% of world-wide production is grown in temperate zones above 30° Latitude N., where three of the four major producers (India, USA, China and Pakistan) are located. Cotton when found in nature is a woody perennial tree, which has been domesticated and converted to an annual crop; it is planted and harvested annually and is not considered to have weedy characteristics. Seeds are the only survival structures. In most temperate cotton growing areas some of the seed remaining in the field following harvest and cultivation may germinate in the autumn if conditions are favorable. The seeds not germinating are likely to rot and die. In cotton growing regions with mild and dry winters, cottonseed may over-winter and germinate the following spring. These cotton volunteers can be easily controlled by current agronomic practices including cultivation and the use of appropriate herbicides such as atrazine, bromoxynil or paraquat.

Cotton is cultivated by the Member States of Greece, Spain, Bulgaria and, to a lesser extent, Portugal. No wild relatives of cotton have been reported in Europe.

H. Taxonomic status, common name, point of collection or acquisition, and characteristics of the donor organism(s) related to biosafety

GHB614xLLCotton25xMON 15985 cotton has been obtained by conventional crossing of lines containing the single events: GHB614, LLCotton25 and MON 15985. No new genetic modification was used for the development of GHB614xLLCotton25xMON 15985 cotton.

In case of GHB614 cotton, the T-DNA region of plasmid pTEM2 that was inserted into the cotton genome contains a single 2mEPSPS expression cassette, "Ph4a748At-intron1h3At-TPotpC::*2mepsps*::3´histonAt".

The *2mepsps* gene originates from genomic DNA of the common food crop plant, maize.

Taxonomic status:	*Zea mays* L.
Common name:	maize
Point of collection or acquisition	Lebrun *et al.*, 2003. US Patent US6566587B1 (20-MAY-2003).
Characteristics related to biosafety	Risk Class 1(lowest risk class)

The high level constitutive histone H4 promoter (Ph4a748At), histone intron (intron1 h3At) and polyadenylation signal (3'histonAt) were all isolated from the plant *Arabidopsis thaliana.*

Taxonomic status:	*Arabidopsis thaliana*
Common name:	*Arabidopsis thaliana*
Point of collection or acquisition	Chaboute *et al.*, 1985. Plant Mol. Biol. **8**:179-191 Chaubet *et al.*, 1992. J. Mol. Biol. **225**:569-574
Characteristics related to biosafety	Risk Class 1 (lowest risk class) for human, animal and plant

The optimized transit peptide (TPotpC), which targets the mature 2mEPSPS protein to plastids, contains sequences from the common food crop plants, maize and sunflower

Taxonomic status:	*Zea mays* L.
Common name:	maize
Taxonomic status:	*Helianthus annuus* L.
Common name:	sunflower
Point of collection or acquisition	Lebrun *et al.*, 1997. US Patent US5510471 (23-APRIL-1996).
Characteristics related to biosafety	Risk Class 1 (lowest risk class)

In the case of LLCotton25 event, the T-DNA region of plasmid pGSV71 that was inserted into the cotton genome contains a single PAT expression cassette, P35S3::*bar*::3´nos.

The *bar* gene was isolated from genomic DNA of *Streptomyces hygroscopicus*, a common soil microbe, not known to be a human, animal or plant pathogen.

Taxonomic status:	*Streptomyces hygroscopicus*
Common name:	*Streptomyces hygroscopicus*
Point of collection or acquisition	Thompson *et al.*, 1987. The EMBO Journal, **6**:2519-2523.
Characteristics related to biosafety	Risk Class 1 (lowest risk class)

The high level constitutive P35S3 promoter was isolated from the plant pathogen Caulifower Mosaic Virus:

Taxonomic status:	Cauliflower Mosaic Virus
Common name:	Cauliflower Mosaic Virus (CaMV)
Point of collection or acquisition	Odell at al., 1985. Nature **313**:810-812
Characteristics related to biosafety	Risk Class 2 (moderate risk)

In the terminating signal of the chimeric *bar* gene, the polyadenylation signal is provided by the 3'untranslated region of the nopaline synthase gene of *Agrobacterium*.

Taxonomic status:	*Agrobacterium tumefaciens*
Common name:	*Agrobacterium tumefaciens*
Point of collection or acquisition	Depicker *et al*. (1982). Journal of Molecular and Applied Genetics **1** : 561-573
Characteristics related to biosafety	Risk Class 1 (lowest risk class)

MON 15985 cotton was produced by the particle acceleration transformation of MON 531 event, with the integration into the MON 531 cotton genome of the MON 15947 insert that contains the PV-GHBK11L DNA fragment.

The MON 531 cotton event was previously produced by *Agrobacterium tumefaciens* mediated transformation with the integration of the MON 531 insert into the *G. hirsutum* genome.
The MON 531 insert contains two expression cassettes: Cry1Ac (P-e35S::CS-cry1Ac::T-7S) and NPTII (P35S::CS-*nptII*::T-nos). In addition, MON 531 contains some sequences of the origin of replication for *Agrobacterium* (*ori*-V).

P-e35S is a 0.61Kb long sequence containing the promoter and leader for the cauliflower mosaic virus (CaMV) 35S RNA containing the duplicated enhancer region. This modification was made to enhance the activity of this promoter in plants.

Taxonomic status:	Cauliflower Mosaic Virus
Common name:	Cauliflower Mosaic Virus (CaMV)
Point of collection or acquisition	Kay et al., 1987. Science 236:1299-1302
Characteristics related to biosafety	Risk Class 2 (moderate risk)

The *cry1Ac* gene codes for a *Bt*-toxin, which confers resistance to lepidopteran pests.

Taxonomic status:	*Bacillus thurigiensis* subsp. *kumamotoensis*
Common name:	*Bacillus thurigiensis* subsp. *Kumamotoensis*
Characteristics related to biosafety	Risk Class 2 (moderate risk)

In the terminating sequence of the chimeric *cry1Ac* gene the polyadenylation signal is provided by the 3´ untranslated region of the 7S seed storage protein gene (7S 3') derived from soybean.

The coding sequence of the neomycine phosphotransferase gene from the bacteria *Escherichi coli* is commonly used as selection marker and is under the control of the P35S3 promoter. The polyadenylation signal is provided by the 3'untranslated region of the nopaline synthase gene of *A. tumefaciens*. In addition, MON 531 contains some sequences of the origin of replication for *Agrobacterium* (*ori*-V).

The gene *aad*, which was derived from *Escherichia coli*, encodes the bacterial selectable marker enzyme 3''(9)-O-aminoglycoside adenyltransferase (AAD), which confers resistance to the antibiotics spectinomycin and streptomycin, and facilitates the selection of bacteria containing the plasmid in the initial steps of transforming the cotton tissue. The promoter that drives the expression of the aad gene was derived from bacteria hence the encoded protein is not expressed in plants derived from cotton event MON 15985.

The MON 15947 insert contains two adjacent plant gene expression cassettes: the gene of interest, *cry2Ab2* (P-e35S-L-Hsp70-TS-ctp2::CS-*cry2Ab2*::T-nos), and the marker gene *uidA* (P-e35S::CS-*uidA*::T-nos) which encodes the GUS protein.

The expression of the modified *cry2Ab2* gene originally isolated from *B. thurigiensis* is driven by the enhanced P-e35S promoter and which is fused to the 5' untranslated leader sequence of the heat shock protein 70 (*Hsp70*) from Petunia (*Petunia hybrida*) and to the N-terminal chloroplast transit peptide (*CTP2*) of the *epsps* gene from *A. thaliana*. At the 3' of the *cry2Ab2* gene the signal for the mRNA polyadenylation is given by the 3' unstranslated region of the nopaline synthase gene of *A. tumefaciens*.

Cry2Ab2

Taxonomic status:	*Bacillus thurigiensis* subsp. *kumamotoensis*
Common name:	*Bacillus thurigiensis* subsp. *Kumamotoensis*
Characteristics related to biosafety	Risk Class 2 (moderate risk)

The gene *uidA*, the coding sequence for the ß-D-glucuronidase protein, is regulated by the enhanced P-e35S promoter at the 5' and the 3' unstranslated region of the nopaline synthase gene of *A. tumefaciens* at the 3'.

I. Approved uses of the LMO

GHB614xLLCotton25xMON 15985 cotton has been notified for cultivation and commercial use in USA.
Applications for the use of GHB614xLLCotton25xMON 15985 for food, feed and industrial uses have been submitted to Australia and New Zealand, Canada, Japan, Korea and Mexico.

J. A risk assessment report consistent with Annex II to Directive 2001/18/EC

A risk assessment in accordance with Annex II to Directive 2001/18/EC has been included in the application for the authorization of glyphosate and glufosinate ammonium herbicides tolerant and insect resistant genetically modified cotton GHB614xLLCotton25xMON 15985 for food and feed uses, and import and processing in accordance with articles 5 and 17 of Regulation (EC) N° 1829/2003.

K. Suggested methods for the safe handling, storage, transport and use, including packaging, labelling, documentation, disposal and contingency procedures, where appropriate

The present application only concerns import of grain derived from GHB614xLLCotton25xMON 15985 cotton for food and feed and excludes cultivation. The commercial production will be in the USA and will enter the European Union (EU) as import of commodity cotton seed under different stages of processing. The only foreseeable chance for GHB614xLLCotton25xMON 15985 to outcross to cotton in Europe would be the unlikely case of imported seed spilled in transit, growing and

flowering if plants established within 12 meters of cultivated cotton. However seeds of G. hirsutum typically require some form of treatment to ensure adequate germination: heat treatment and a sulphuric acid delinting treatment to remove fuzz or linters from the seed coat (the delinted seed is also known as 'black' seed) and most of the seed will be imported as non viable seed. Seed cotton and fuzzy seed germinate poorly, probably because the lint and linters attached to the seed coat limit contact with soil thereby inhibiting imbibing soil moisture. Seeds that may escape during transport do not give rise to persistent populations due to the seed treatment requirements. The need for significant moisture also prohibits growth of escapes in many locations. Even in areas with significant rainfall, escaped cotton has not been able to establish due to its poor colonizing ability

GHB614xLLCotton25xMON 15985 would only have a competitive advantage under pressure of selection in an area treated with glyphosate or glufosinate ammonium herbicides, or in conditions of a severe lepidopteran pest infestation and could establish in the environment modifying the biodiversity. Furthermore, it might transfer the traits via pollen flow to other cultivated cottons (no wild relative of cotton is present in Europe) in the vicinity. Cotton arising from spillage can be controlled using conventional herbicides such as 2,4-D, dicamba, atrazina or paraquat for example.

Thus the potential interactions of imported GHB614xLLCotton25xMON 15985 cotton commodities with the biotic environment are extremely limited and no different from the import of traditional cotton commodities.

It is the responsibility of the importer to follow the documentation requirements of the Cartagena Protocol on Biosafety and Regulation (EC) 1946/2003. Operators, commercialising materials containing or derived from GHB614xLLCotton25xMON 15985 cotton need to label the products in accordance with Regulation (EC) 1830/2003.