PART II

Request for Authorization

in accordance with the articles 5 and 17 of Regulation (EC) 1829/2003 GM Food and GM Feed

Glyphosate and glufosinate ammonium-tolerant and insect-resistant GM cotton GHB614xLLCotton25xMON 15985, and the subcombination LLCotton25xMON 15985 for food and feed uses, and for import and processing

A. GENERAL INFORMATION

1. Details of application

a) Member State of application: The Netherlands
b) Application number: Not available at the date of application
c) Name of the product (commercial and other names): GHB614xLLCotton25xMON 15985 cotton also referred in this application as GTxLLxB2 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 GTxLLxB2 cotton. The unique identifier assigned to GTxLLxB2 cotton is: BCS-GHØØ2-5 x ACS-GHØØ1-3 x MON-15985-7
d) Date of acknowledgement of valid application: Not available at the date of application.

2. Applicant

a) Name of applicant: Bayer CropScience AG.		
b) Address of applicant:		
Bayer CropScience AG Alfred-Nobel-Strasse 50 D - 40789 Monheim am Rhein Germany	represented by	Bayer BioScience NV Technologiepark 38 B-9052 Gent Belgium
c) Name and address of the person established in the Community who is responsible for the placing on the market, whether it be the manufacturer, the importer or the distributor, if different from the applicant (Commission Decision 2004/204/EC Art 3(a)(ii)): GTxLLxB2 cotton will be imported and processed in the EU by the same groups who currently import, process and distribute commodity cottonseed		

3. Scope of the application

☒ GM plants for food use

☒ Food containing or consisting of GM plants

☒ Food produced from GM plants or containing ingredients produced from GM plants

☒ GM plants for feed use

☒ Feed containing or consisting of GM plants

☒ Feed produced from GM plants

☒ Import and processing (Part C of Directive 2001/18/EC)

☐ Seeds and plant propagating material for cultivation in Europe (Part C of Directive 2001/18/EC)

4. Is the product being simultaneously notified within the framework of another regulation (*e.g.* Seed legislation)?

Yes ☐	No ☒
If *yes*, specify	

5. Has the GM plant been notified under Part B of Directive 2001/18/EC and/or Directive 90/220/EEC?

Yes ☐	No ☒
If *no*, refer to risk analysis data on the basis of the elements of Part B of Directive 2001/18/EC An environmental risk assessment for GTxLLxB2 cotton has been carried out in accordance with Annex II to Directive 2001/18/EC and Commission Decision 2002/623/EC and is described in point D.9 below.	

6. Has the GM plant or derived products been previously notified for marketing in the Community under Part C of Directive 2001/18/EC or Regulation (EC) 258/97?

Yes ☐	No ☒
If *yes*, specify: -	

7. Has the product been notified in a third country either previously or simultaneously?

Yes ☒	No ☐
If *yes*, specify: Authorisation requested for cultivation and commercial use in USA Authorisation for food, feed and industrial uses in Australia and New Zealand, Canada, Japan, Korea and Mexico	

8. General description of the product

a) Name of the recipient or parental plant and the intended function of the genetic modification:

GTxLLxB2 cotton has been obtained by conventional crossing between three genetically modified cotton parental lines: GHB614, LLCotton25 and MON 15985. The parental lines were obtained by genetic modification of *Gossypium hirsutum*. GHB614, LLCotton25 and MON 15985 were conventionally bred by introgression into an array of varieties belonging to the species *G. hirsutum*.

No new genetic modification was used for the development of GTxLLxB2 cotton.

The combined event GTxLLxB2 inherited the following traits from the parental lines: tolerance to glyphosate herbicides, tolerance to glufosinate ammonium herbicides and resistance to certain lepidopteran insects respectively.

b) Types of products planned to be placed on the market according to the authorisation applied for:

Two different types of products are planned to be placed on the market: 1) grain from GTxLLxB2 and 2) cottonseed products derived from event GTxLLxB2.

1) GTxLLxB2 grain will be imported, processed and distributed in the European Union similar to current cottonseed usage (food, feed and industrial uses) excluding cultivation.

2) Cottonseed products derived from event GTxLLxB2 (cottonseed oil, meal and linters) will be imported in the EU, similar to current usage of products derived from cottonseed (food, feed and industrial uses).

c) Intended use of the product and types of users:

GTxLLxB2 grain and cottonseed products derived from event GTxLLxB2 will be imported in the EU from the major cotton growing areas as a commodity and will be used for downstream purposes for food, feed and industrial products by users identical to current conventional cottonseed and cottonseed cotton importers.

d) Specific instructions and/or recommendations for use, storage and handling, including mandatory restrictions proposed as a condition of the authorisation applied for:

No mandatory restrictions for use, storage and handling are proposed as a condition of the authorisation. All standard practices applicable to cotton today remain adequate for the handling of GTxLLxB2 cotton.

When GTxLLxB2 cotton is placed on the EU market, the labelling and traceability requirements according to Regulation (EC) N° 1829/2003 and Regulation (EC) N° 1830/2003 will apply.

e) Any proposed packaging requirements:

No specific packaging requirements are necessary.

f) A proposal for labelling in accordance with Articles 13 and Articles 25 of Regulation ((EC) 1829/2003. In the case of GMOs, food and/or feed containing or consisting of GMOs, a proposal for labelling has to be included complying with the requirements of Article 4, B(6) of Regulation (EC) 1830/2003 and Annex IV of Directive 2001/18/EC:

GTxLLxB2 cotton does not harbour characteristics that require specific labelling. Hence, no additional labelling is proposed other than the GM labelling requirements under regulations (EC) N° 1829/2003 and N° 1830/2003

g) Unique identifier for the GM plant (Regulation (EC) 65/2004; does not apply to applications concerning only food and feed produced from GM plants, or containing ingredients produced from GM plants): BCS-GHØØ2-5 x ACS-GHØØ1-3 x MON-15985-7

h) If applicable, geographical areas within the EU to which the product is intended to be confined under the terms of the authorisation applied for. Any type of environment to which the product is unsuited:

Not applicable because restrictions are not necessary

9. Measures suggested by the applicant to take in case of unintended release or misuse as well as measures for disposal and treatment

The majority of imported cotton commodities will be processed products from different levels of downstream processing without the ability for natural reproduction. Viable cottonseed will be imported in small quantities only. The safety profile in terms of human and animal health and environmental impact of GTxLLxB2 seeds is identical to that of conventional cottons and do not constitute a hazard.

The case of accidental spillage of non-processed GTxLLxB2 cotton, in transit or at the processing facility, has been considered in the risk assessment and foreseen in the post market monitoring plan (see paragraph 11.4).

B. INFORMATION RELATING TO THE RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANTS

1. Complete name

a) Family name:	*Malvaceae*
b) Genus:	*Gossypium*
c) Species:	*hirsutum*
d) Subspecies:	Not applicable
e) Cultivar/breeding line or strain:	GHB614, LLCotton25, MON 15985
f) Common name:	cotton

2 a. Information concerning reproduction

(i) Mode(s) of reproduction

Cultivated cotton is propagated by seeds. In the absence of insect pollinators, cotton is a self-pollinator, but cross-pollination may take place when pollinators are present.

(ii) Specific factors affecting reproduction

Although natural crossing can occur, cotton is normally considered to be a self-pollinating crop. Cotton pollen is very large, heavy and sticky, and thus not wind-borne. The pollen can be transferred by various insects like bumble bees (Bombus ssp.) and honey bees (Apis mellifera). The frequency of cross-pollination varies with the insect pollinator population. All the factors reducing the density of

pollinators such as the use of insecticides, or increased air humidity as the result of irrigation will essentially limit the extent of cross-pollination.
The main abiotic environmental factors affecting cotton reproduction also determine the areas of cotton production. They are: high light intensity and optimal temperature profiles, such as a) active vegetative growth range: 15 - 38 °C, b) accumulated heat GD 15.5°C need: 1,200 units, c) number of frost free days: 200, d) rapid and consistent spring warming pattern.

(iii) Generation time

The cultural cycle for cotton ranges from less than 100 days, to 200 growing days from seedling emergence to maturity depending on the variety. Rainfall, temperature, sunshine and spring warming, all have an impact on optimal growth.

2 b. Sexual compatibility with other cultivated or wild plant species

There are no identified non-cotton plants that are sexually compatible with cultivated cotton varieties presently found in the EU.
Pre-zygotic, and *post-zygotic* barriers greatly limit the sexual compatibility of *G. hirsutum* and *G. barbadense* with other plant species in the Gossypiae tribe. In addition plants of the *Gossypium* genus are not native to Europe. Several members of the Malvaceae family are cultivated as ornamental plants (*e.g. Hibiscus rosa-sinensis*) or vegetables *(e.g. Abelmoschus esculentus*–okra), but hybridisation experiments of these species with *Gossypium* spp. failed or resulted in sterile seeds.
G. hirsutum and *G. barbadense*, allotetraploid species that combine the AADD genomes, will hybridise only with other tetraploid members of the *Gossypium* genus including *G. tomentosum, G. darwinii, G. mustelinum, G. hirsutum, G. barbadense* and *G. lanceolatum*, which species are not known to have a habitat in Europe

3. Survivability

a) Ability to form structures for survival or dormancy

Cotton is cultivated annually and cannot survive without human assistance. Seeds are the only vegetative structure for survival. Some wild forms may produce “hard seeds” that, upon drying, become impermeable to water and suffer delayed germination. However this trait is undesirable agronomically and has been largely bred out from modern cultivars through breeding and selection. Cultivated cotton does not produce seeds which can persist in the environment for long periods of time, furthermore cotton seed lacks the ability to develop dormancy.

b) Specific factors affecting survivability

The main factors affecting survivability of cotton are related to soil microclimate such as temperature and humidity. If planted in moist soil before the soil temperature reaches 15 °C, the cotton seed is likely to rot and die.

4. Dissemination

a) Ways and extent of dissemination

Two differentiated reproductive structures are suitable for the dispersal of cotton genes in the environment:

1. Seed dispersal. It could occur during transport, at planting and essentially before and during harvest.
2. Pollen dispersal. A number of studies conclude that when out-crossing occurs it is principally located around the pollen source and decreases significantly with distance.

b) Specific factors affecting dissemination

Seeds dispersal: Cotton seed has no structural modifications to facilitate transfer by animals.

Dissemination is mainly due to human activity.

Pollen dispersal in cotton shows correlation with insect prevalence. Proximity of more attractive vegetation, climate and insect management will essentially limit the extent of cross-pollination.

5. Geographical distribution and cultivation of the plant, including the distribution in Europe of the compatible species

Plants of the tribe *Gossypiae* originated in the tropics and subtropics. Wild species of the tribe are extremely sensitive to photoperiod conditions and do not flower in long day-light regime, therefore they are essentially excluded from temperate climates. In spite of their origin, more than 50 % of cultivated cottons are produced in temperate zone above 30° Latitude N, but they also tend to be plants of the southern hemisphere.
Gossypium hirsutum in its wild form is distributed over the most arid areas of Central America and in the South and North of America, with wild populations that are rare and sporadic, while South America is considered to be the centre of origin of the species *G. barbadense*. Cultivated *G. hirsutum* (Upland or Mexican cotton) represents over 90 % of world-wide production besides one only "New World" tetraploid species, *G. barbadense* (Pima, South American cotton or Egyptian cotton) and two "Old World" diploid species: *G. arboreum* and *G. herbaceum*.
Main cotton producers are China, USA, India, Pakistan, Uzbekistan, Brazil and Turkey.
In Europe, the cultivated cotton is mainly *G. hirsutum*.

6. In the case of plant species not normally grown in the Member State(s), description of the natural habitat of the plant, including information on natural predators, parasites, competitors and symbionts

In the E.U., cotton is commercially grown in Greece and Spain; and is grown in limited surfaces in Bulgaria and Portugal.

7. Other potential interactions, relevant to the GM plant, of the plant with organisms in the ecosystem where it is usually grown, or used elsewhere, including information on toxic effects on humans, animals and other organisms

Cotton is known to interact with other organisms in the ecosystem including a range of beneficial and pestiferous arthropods, bacteria, fungi, nematodes, surrounding weed species, animals and humans.
The crop has been cultivated in Spain and Greece for centuries and has a history of safe use.
The cotton crop was produced for fibre for thousands of years, and was first utilized for food and feed in the 20th century. Cotton is not considered harmful or pathogenic to animals or humans, however the plant does produce a small amount of natural anti nutritional factors such as gossypol and cyclopropenoid fatty acids.
All of the anti-nutritional factors are subject to neutralisation during processing. Free gossypol binds to lysine and other products, and then becomes unavailable to animals. Cyclopropenoid fatty acids are deactivated or removed from the oil by hydrogenation or during deodorization at 230-235°C.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

1. Description of the methods used for the genetic modification

GTxLLxB2 and LLxB2 combined cotton events were developed by the crossing of single parental GHB614, LLCotton25 and MON 15985 lines.
No new genetic modification was introduced in GTxLLxB2 and the subcombination LLxB2.

GHB614, LLCotton25 cotton events were produced by *Agrobacterium tumefaciens* -mediated transformation. MON 15985 cotton was produced by the particle acceleration transformation of MON 531 cotton, which was previously genetically modified via *Agrobacterium*-mediated transformation.

2. Nature and source of the vector used

The following vectors were used for the genetic transformation procedure of the single events:

GHB614

The plasmid vector used for the transformation of GHB614 is pTEM2, which is derived from pGSC1700. Plasmid pTEM2 is part of a binary *A. tumefaciens* system and was specifically designed for the cloning of desirable expression cassettes in cotton.

LLCotton25

The plasmid vector used for the transformation of LLCotton25 is pGSV71, which is derived from pGSC1700. Plasmid pGSV71 is part of a binary *A. tumefaciens* system and was specifically designed for the cloning of desirable expression cassettes in cotton.

MON 15985

The PV-GHBK11L *Kpn*I DNA fragment originating from the plasmid vector PV-GHBK11 was used to generate MON 15985, by the transformation of the MON 531 cotton event.

3. Source of donor DNA, size and intended function of each constituent fragment of the region intended for insertion

Sources, sizes and intended functions of the inserted sequences in GTxLLxB2 combined cotton events, inherited from the respective parental lines are described in tables 1, 2 and 3

GHB614:

Table 1 Source, size and intended function of each constituent fragment of the inserted DNA fragment inherited from GHB614

Genetic Element	Description	Source	Size (base pair)	Intended function
LB	T-DNA left border sequence	*Agrobacterium tumefaciens*	4	T-DNA integration
Ph4a748At	Promoter region of the histone H4 gene	*Arabidopsis thaliana*	1010	High level constitutive expression, especially in the rapidly growing plant tissues
intron1 h3At	Sequence of the first intron of the histone H3.III	*Arabidopsis thaliana*	516	
TPotpC	Transit peptide	*Zea mays* and *Helianthus annuus*	372	Targeting of the protein to the plastids
2mepsps	Coding sequence of the modified 5-enol-pyruvylshikimate-3-phosphate synthase gene	*Zea mays*	1337	Glyphosate herbicide tolerance and selectable marker
3'histon At	3´ untranslated region of the	*Arabidopsis*	742	Transcriptional termination sequence

	histone H4 gene	*thaliana*		
RB	T-DNA right border sequence	*Agrobacterium tumefaciens*	4	T-DNA integration

LLCotton25

Table 2 Source, size and intended function of each constituent fragment of the inserted DNA fragment inherited from LLCotton25

Genetic element	Description	Source	Size (bp[1])	Intended function
RB	T-DNA right border sequence	*Agrobacterium tumefaciens*	2	T-DNA integration
	Polylinker sequence	Synthetic	28	Plasmid cloning site
P35S3	Promoter	Cauliflower Mosaic Virus	1385	High level constitutive expression
bar	Coding sequence of the phosphinotricin acetyltransferase gene	*Streptomyces hygroscopicus*	552	Glufosinate-ammonium herbicide tolerance and selectable marker
	Polylinker sequence	Synthetic	19	Plasmid cloning site
3'nos	3´ untranslated region of the nopaline synthase gene	*Agrobacterium tumefaciens*	261	Transcriptional termination sequence
	Polylinker sequence	Synthetic	51	Plasmid cloning site
LB	T-DNA left border sequence	*Agrobacterium tumefaciens*	21	T-DNA integration

[1] bp: base pair

MON 15985

Table 3 Source, size and intended function of each constituent fragment of the inserted DNA fragment inherited from MON 15985

Genetic element	Description	Source	Size (kb[1])	Intended function
Genetic elements associated with the MON 531 insert				
3´end of *cry1Ac*	3´ end portion of the coding sequence of the *cry1Ac* gene	*Bacillus thuringiensis*	0.9	Not functional
7S 3´	3´ untranslated region of the 7S seed storage protein gene	*Glycine max*	0.44	Transcriptional termination sequence
RB	T-DNA right border sequence	*Agrobacterium tumefaciens*	0.15	T-DNA integration
***cry1Ac* expression cassette**				
7S 3´	3´ untranslated region of the 7S seed storage protein gene	*Glycine max*	0.44	Transcriptional termination sequence
cry1Ac	Coding sequence of the *cry1Ac*	*Bacillus thuringiensis*	3.54	Insect resistance
e35S	Promoter with duplicated enhancer region	Cauliflower mosaic virus	0.62	High level constitutive expression
***aad* gene**				
aad	Coding sequence for the aminoglycoside-modifying enzyme	Bacterial transposon Tn*7*	0.79	Resistance to spectinomycin and streptomycin (selectable marker)
***nptII* expression cassette**				
3'nos	3´ untranslated region of the nopaline synthase gene	*Agrobacterium tumefaciens*	0.24	Transcriptional termination sequence
nptII	Coding sequence of the neomycin phosphotransferase gene	Bacterial transposon Tn*5*	0.97	Resistance to kanamycin (selectable marker)
35S	Promoter	Cauliflower mosaic virus	0.32	High level constitutive expression
ori-V	Origin of plasmid replication	*Agrobacterium tumefaciens*	0.39	Maintenance of plasmid in *Agrobacterium*

[1] kb: kilobase pairs

Table 3 Source, size and intended function of each constituent fragment of the inserted DNA fragment inherited from MON 15985 (continued)

Genetic element	Description	Source	Size (kb[1])	Intended function
Genetic elements associated with the MON 15947 insert				
uidA expression cassette				
e35S	Promoter with duplicated enhancer region	Cauliflower mosaic virus	0.6	High level constitutive expression
uidA	Coding sequence for the ß-D-glucuronidase protein	*E.coli*	1.8	Selectable marker
3'nos	3´ untranslated region of the nopaline synthase gene	*Agrobacterium tumefaciens*	0.26	Transcriptional termination sequence
cry2Ab2 expression cassette				
e35S	Promoter with duplicated enhancer region	Cauliflower mosaic virus	0.6	High level constitutive expression
Hsp70	5´ untranslated leader sequence of the heat shock protein 70	Petunia	0.1	Enhancer
CTP2	N-terminal chloroplast transit peptide of the *epsps* gene	*Arabidopsis thaliana*	0.23	Facilitates import of the newly translated proteins into the chloroplast
cry2Ab2	Coding sequence for a synthetic Cry2Ab2 protein	*Bacillus thuringiensis*	1.9	Insect resistance
3'nos	3´ untranslated region of the nopaline synthase gene	*Agrobacterium tumefaciens*	0.26	Transcriptional termination sequence

[1] kb: kilobase pairs

D. INFORMATION RELATING TO THE GM PLANT

1. Description of the trait(s) and characteristics which have been introduced or modified

The following traits were inherited in the GTxLLxB2 cotton from the single events GHB614, LLCotton25 and MON 15985:

- ***Tolerance to glyphosate herbicides***

The glyphosate herbicide tolerance trait in GTxLLxB2 cotton is inherited from the parental line GHB614 .

GHB614 cotton contains the *2mepsps* gene, which encodes a modified 5-enolpyruvylshikimate 3-phosphate synthase (2mEPSPS). The 2mEPSPS protein confers tolerance to the herbicide glyphosate. Glyphosate is a wide-spectrum herbicide that inhibits the enzyme, 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS), which is involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms. The 2mEPSPS enzyme however is not inhibited by glyphosate and the expression is sufficiently high to provide a good level of specific activity and ensure glyphosate tolerance to event GHB614.

- ***Tolerance to glufosinate ammonium herbicides***

The glufosinate ammonium herbicide tolerance trait in GTxLLxB2 cotton is inherited from the parental line LLCotton25.
LLCotton 25 contains the *bar* gene, a bialaphos resistance gene, isolated from the soil microorganism, *Streptomyces hygroscopicus*. The *bar* gene, when expressed, enables the production of the enzyme, Phosphinothricin-Acetyl-Transferase (PAT) that acetylates L-glufosinate ammonium and thereby confers tolerance to glufosinate ammonium herbicides

- ***Insect resistance***

The insect resistance trait in GTxLLxB2 cotton is inherited from its parental line MON 15985.
MON 15985 has been developed to produce two *Bacillus thuringiensis* crystal proteins, Cry1Ac and Cry2Ab2. They confer protection against feeding damage caused by major lepidopteran insect pests of cotton, including the cotton bollworm (CBW, *Helicoverpa armigera*), tobacco budworm (TBW, *Heliothis virescens*), pink bollworm (PBW, *Pectinophora gossypiella*) and Beet Armyworm (BAW, *Spodoptera exigua*). MON 15985 was produced by stable insertion of the coding sequence for the Cry2Ab2 protein from *Bacillus thuringiensis* subsp. *kurstaki* into the genome of an existing genetically modified cotton, MON 531 (Bollgard cotton), which expresses the Cry1Ac protein.

2. Information on the sequences actually inserted or deleted

a) The copy number of all detectable inserts, both complete and partial

GTxLLxB2 cotton has been obtained by conventional crossing between three genetically modified cotton events: GHB614, LLCotton25 and MON 15985. The analyses of the single events showed that:

GHB614 parental cotton event contains a single copy of the T-DNA region of the pTEM2 plasmid inserted at a single genomic locus. No vector backbone sequences were detected in GHB614 cotton.

LLCotton25 parental cotton event contains a single copy of the pGSV71 plasmid T-DNA, inserted at a single genomic locus. No vector backbone sequences were detected in LLCotton25.

MON 15985 parental cotton event contains two different DNA inserts of MON 531 and MON 15947 origins that integrated at two different loci. MON 15985 does not contain any detectable plasmid backbone sequences resulting from both transformations

The intactness and stability of the inserts and their flanking regions inherited by GTxLLxB2 cotton from the individual parental events GHB614, LLCotton25 and MON 15985 was demonstrated by a complete and detailed Southern blot analysis.

Identical Southern hybridization patterns were observed for GTxLLxB2 cotton compared to GHB614, LLCotton25 and MON 15985 parents, thereby confirming the intactness and stability of the parents' inserted sequences and their flanking regions in GTxLLxB2 cotton.

b) The organization of the inserted genetic material at the insertion site

As described in Section D.2.a), Southern blot analyses confirmed the equivalent organization of the inherited DNA sequences present in GTxLLxB2 cotton to those present in each single GHB614, LLCotton25 and MON 15985 parental lines.

c) Chromosomal location(s) of insert(s) (nucleus, chloroplasts, mitochondria, or maintained in a non-integrated form), and methods for its determination

Not applicable, the molecular analysis indicates that there are no deleted regions in the parental lines or in GTxLLxB2 and LLxB2 combined cotton events

d) In case of deletion(s), size and function of the deleted region(s)

Not relevant

3. Information on the expression of the insert

a) Information on developmental expression of the insert during the life cycle of the plant

Levels of 2mEPSPS, PAT, Cry1Ac, Cry2Ab2, NptII and GUS proteins were measured in leaves, squares and seed (kernels) plant tissues of GTxLLxB2 cotton, and compared to the GHB614, LLCotton25, MON and 15985 parental lines.
Enzyme Linked Immunosorbent Assay (ELISA) with specific antibodies was used to quantify expression of 2mEPSPS, PAT, Cry1Ac, Cry2Ab2 and NPTII proteins. Levels of the GUS protein were measured by a quantitative GUS protein-specific enzymatic assay.
Material for these tests was collected from plants grown in greenhouse conditions.
The protein expression levels of 2mEPSPS, PAT, Cry1Ac, Cry2Ab2, GUS and NptII measured in leaves, squares and seed plant tissues of GTxLLxB2 are comparable with the levels observed in GHB614, LLCotton25 and MON 15985 parental lines thereby confirming that the combination of the events did not have an impact in the protein expression levels of 2mEPSPS, PAT, Cry1Ac, Cry2Ab2 and NPTII.

b) Parts of the plant where the insert is expressed

As described in Section C.3., two constitutive promoters, CaMV 35S and *Arabidopsis* histon H4, regulate expression of the *bar*, *cry1Ac*, *cry2Ab2*, *uidA*, *nptII* and *2mepsps* genes, respectively, with high activity in green tissues and seeds.

As described in detail in Section D.3.a., 2mEPSPS, PAT, Cry1Ac, Cry2Ab2, GUS and NPTII are expressed in leaves, squares and seed plant tissues of GTxLLxB2 cotton at levels comparable to those observed in the respective GHB614, LLCotton25 and MON 15985 parent lines.

4. Information on how the GM plant differs from the recipient plant in

a) Reproduction

The traits of glyphosate, glufosinate ammonium herbicides tolerance and insect resistance of GTxLLxB2 cotton had no effect on the mode and rate of seed reproduction which was found to be the same as for conventional cotton, as observed in field trials in USA during the 2008 growing season.

b) Dissemination

Two developmental stages in cotton are susceptible to dispersal: pollen and seed. No differences in dissemination capacity have been observed between GTxLLxB2 and conventional cotton. Studies show that the genetic modification did not change any characteristics of the cotton that could impact dissemination:
- morphology, viability and survival rate of the pollen between the GTxLLxB2 cotton, the parental genotypes and the non-transgenic counterpart examined in the study;
- no difference in seed morphology or fecundity measured as number of seed per boll and 100 seed weight;
- no difference in germination/stand count, seedling vigour or dormancy as measured by standard laboratory cotton seed physiology tests

c) Survivability

The characteristics of GTxLLxB2 cotton that could have an impact on survivability, such as germination rate and vigour remain unchanged when compared to the GHB614, LLCotton25, MON 15985 parents and the conventional non-GM counterpart.

d) Other differences

The only differences are the resistance to insect pests of the Lepidoptera family, and the tolerance to glufosinate-ammonium and glyphosate herbicides.

5. Genetic stability of the insert and phenotypic stability of the GM plant

The molecular, genetic and phenotypic stability of GHB614, LLCotton25 and MON 15985 has been demonstrated by Southern blot analyses performed on multiple generations, protein expression studies and by assessment of agronomic performance of plants in multiple field trials.

Southern blot analyses demonstrated the intactness and stability of GHB614, LLCotton25 and MON 15985 transgenic sequences in GTxLLxB2 cotton (D.2.a).

It was demonstrated that 2mEPSPS, PAT, Cry1Ac, Cry2Ab2, NPTII and GUS proteins present in the leaves, squares and seed tissues of the parents GHB614, LLCotton25 and MON 15985, are present in the leaves, squares and seed tissues of the GTxLLxB2 cotton at essentially the same levels, thereby confirming the absence of interactions between transgene loci on a protein level. (D.3).

Comparative assessment of phenotypic and agronomic characteristics of GTxLLxB2 and the parental lines GHB614, LLCotton25, MON 15985 in field trials conducted at different locations in the USA in the 2008 growing season (Section D.7.5.) demonstrated that GTxLLxB2 cotton is equivalent to its parents with regard to phenotypic characteristics and agronomic performance except for the combined traits.

In conclusion, the GTxLLxB2 cotton generated by conventional crossing of the GHB614, LLCotton25 and MON 15985 parental lines is genetically and phenotypically stable.

6. Any change to the ability of the GM plant to transfer genetic material to other organisms

a) Plant to bacteria gene transfer

Since GTxLLxB2 and LLxB2 combined events were produced by conventional crossing of three events, no change in ability to transfer genetic material to other organisms is expected in the combined product.
In addition, as cultivation is not within the scope of this application, transfer of genetic material to other organisms is a very remote possibility.

b) Plant to plant gene transfer

Analysis of the basic parameters relating to reproductive fitness of GTxLLxB2 cotton was performed in field trial studies in the USA during the 2008 growing season. For all parameters evaluated, GTxLLxB2 cotton was found to be unchanged compared to the conventional cotton, thereby confirming that the potential for gene transfer from GTxLLxB2 to cultivated cotton and/or wild relatives is the same as with any commercially available cotton.

7. Information on any toxic, allergenic or other harmful effects on human or animal health arising from the GM food/feed

7.1 Comparative assessment

A comparative assessment for compositional and nutritional equivalence was performed on GTxLLxB2 cotton grain collected from a field trial carried out at seven locations in the USA during the 2008 growing season. The comparative assessment was conducted to determine whether GTxLLxB2 cotton grain is compositionally and nutritionally equivalent to grain from the non GM comparator FiberMax 958 with the comparable genetic background and to grain from GHB614, LLCotton25 and MON 15985 parental lines, and whether GTxLLxB2 cotton is equivalent to the chosen comparators with regard to phenotypic and agronomic plant performance.

The results of this comparative analysis of GTxLLxB2 cotton grain from field trials in USA demonstrates that grain from GTxLLxB2 cotton is compositionally and nutritionally equivalent to grain from the non GM comparator FM958 and GHB614, LLCotton25 and MON 15985 parental lines, and spraying with glufosinate ammonium and glyphosate herbicides, does not have an effect on the nutrient composition of GTxLLxB2 cotton grain. The GTxLLxB2 phenotypic appearance and its agronomic performance did not differ in comparison with the commercial varieties and parental lines.

7.2 Production of material for comparative assessment

a) Number of locations, growing seasons, geographical spread and replicates

The comparative assessment of cotton event GTxLLxB2 was performed during field trials carried out in seven different locations, representing typical cotton growing regions of the south-eastern United States, over 2008 growing season. The field trial design was a Randomized Complete Block Design with three repetitions and six different treatment regimens

b) The baseline used for consideration of natural variations

A range of values to be expected for each component was established from published literature, as well as from the values for the reference non GM counterpart variety FiberMax958, and the three parental lines GHB614, LLCotton25, MON 15985.

7.3 Selection of material and compounds for analysis

The compounds which were selected for compositional and nutritional analyses of GTxLLxB2 cotton grain comprise the important basic nutrients of cotton as defined by the OECD. These are proximates (protein, fat, ash, carbohydrates, and moisture), amino acids, fatty acids, micronutrients, such as vitamins and minerals (alpha-tocopherol, calcium, phosphorus, magnesium, potassium, iron, zinc) and anti-nutrients, such as gossypol and cyclopropenoid fatty acids.

7.4 Agronomic traits

Agronomic performance evaluation of GTxLLxB2 cotton was carried out at 7 locations in the USA during the 2008 growing season. The agronomic evaluations included a detailed phenotypic analysis based upon plant variety description, agronomic performance evaluations common to yield trials, including disease resistance evaluations and agronomic practice evaluations.
Overall this study demonstrates that the agronomic characteristics of GTxLLxB2 cotton are comparable to the near isogenic non GM cotton variety FM958 and to the parental lines.

7.5 Product specification

Commercial varieties derived from GTxLLxB2 and LLxB2 varieties belong to the species, *Gossypium hirsutum* L., and are distinguished from other cotton only by tolerance to the herbicides, glyphosate (2mEPSPS protein) and/or glufosinate ammonium (PAT protein), and/or resistance to lepidopteran pests imparted by the expression of the *Cry1Ac* and *Cry2Ab2* genes.

GTxLLxB2 will be introgressed into an array of elite varieties

No new genetic modification was used for the development of GTxLLxB2 cotton. The four primary products coming from cottonseed processing are oil, meal , hulls and linters.

As discussed in detail in this application, GTxLLxB2 cotton is as safe as and as nutritious as commercially available cotton and therefore, the specification of food and animal feed from GTxLLxB2 cotton is equivalent to that of food and animal feed from commercially available cotton.

7.6 Effect of processing

The same production processes applied to traditional cottonseed will be used for GTxLLxB2 and LLxB2 cottonseed. GTxLLxB2 and LLxB2 cotton will be grown using the agronomic practices of the region of production and the grain will harvested, transported, stored and processed using the same processes as used for any other cotton in commerce.

7.7 Anticipated intake/extent of use

The intake of cottonseed products in the diet of the European Union (EU27) is not anticipated to change with the introduction of GTxLLxB2 and LLxB2 cotton varieties. Cottonseed and cottonseed products derived from GTxLLxB2 and LLxB2 varieties are not different in quality or nutritional composition from the cottonseed products now consumed. No change in the use patterns for cotton is anticipated. No potential dietary and nutritional impacts have been identified for cottonseed and cottonseed products derived from GTxLLxB2 and LLxB2 varieties.

7.8 Toxicology

7.8.1 Safety assessment of newly expressed proteins

GTxLLxB2 and LLxB2 combined events cotton were developed by conventional crossing of the GHB614, LLCotton25 and MON 15985 lines. No new genetic modification was introduced in GTxLLxB2 and LLxB2 cottons and therefore, there are no newly expressed proteins in GTxLLxB2 and LLxB2 cottons other than the ones already assessed as safe in the case of GHB614, LLCotton25 or that are waiting a positive EFSA evaluation as MON 15985.

7.8.2 Testing of new constituents other than proteins

Not applicable since no new constituents other than proteins are present in GTxLLxB2 cotton

7.8.3 Information on natural food and feed constituents

As described in detail in Section D.7.3., natural constituents of cotton have not been changed in GTxLLxB2 cotton.

7.8.4 Testing of the whole GM food/feed

GTxLLxB2 cotton and the subcombination LLxB2 were developed by the conventional crossing of single parental lines GHB614, LLCotton25 and MON 15985. No new genetic modification was

introduced in the GTxLLxB2 cotton and the subcombination LLxB2.

The single cotton events LLCotton25 and GHB614 have been previously assessed as safe (EFSA Opinion, 2006; EFSA Opinion, 2009). The application for authorization to place on the market MON 15985 cotton in the European Union, according to Regulation (EC) No 1829/2003 on genetically modified food and feed, was acknowledged as valid by EFSA on June 5, 2008 and is currently under EFSA scientific risk assessment.

In addition, and as described in Section D.3., the 2mEPSPS, PAT, Cry1Ac, Cry2Ab2, GUS, and NPTII protein expression levels in seed, leaf and square plant tissues of GTxLLxB2 are similar to those observed in the relevant parental events, thereby confirming that the combination of the GHB614, LLCotton25 and MON 15985 inserts in GTxLLxB2 cotton did not have an impact on the 2mEPSPS, PAT, Cry1Ac, Cry2Ab2, GUS, and NPTII protein expression levels.

Furthermore, and as described in Section D.7.3 of this application, compositional analysis has confirmed that GTxLLxB2 cottonseed is compositionally and nutritionally equivalent to cottonseed of the non-GM counterpart and of conventional cotton.

In conclusion, GTxLLxB2 cotton and the subcombination LLxB2 are as safe as and as nutritious as any other commercially available cotton for human food and animal feed use and no further testing of the whole GM food/feed is considered necessary.

7.9 Allergenicity

7.9.1 Assessment of allergenicity of the newly expressed protein

GTxLLxB2 and LLxB2 cottons were developed by conventional crossing of the single parental lines GHB614, LLCotton25 and MON 15985. No new genetic modification was introduced in the GTxLLxB2 cotton and therefore, there are no newly expressed proteins in GTxLLxB2 cotton other than the ones already assessed as safe in the case of GHB614, LLCotton25 and MON 15985.

The absence of any allergenic potential of the proteins associated with the inherited genes *2mepsps* and *bar*, expressed in the parents GHB614 and LLCotton25 respectively has previously been demonstrated. The application for authorization to place on the market MON 15985 cotton in the European Union, according to Regulation (EC) No 1829/2003 on genetically modified food and feed, was acknowledged as valid by EFSA on June 5, 2008 and is currently under EFSA scientific risk assessment.

7.9.2 Assessment of allergenicity of the whole GM plant or crop

Cotton (*Gossypium spp*.) is not considered an allergenic food crop.
A consideration of specific food safety issues did not identify food allergenic potential as one outcome that would cause concern for human consumption. Edible oils that are refined, bleached and deodorised do not appear to pose a risk to allergic individuals, as they contain virtually no proteins. Literature to date on cottonseed oil validates this theory: the absence of water-soluble allergens in cottonseed oil is correlated with no clinical allergy observations after consumption of cottonseed oil. Therefore, no allergic reaction is expected from its current use pattern in the case of GTxLLxB2

7.10 Nutritional assessment of GM food/feed

7.10.1 Nutritional assessment of GM food

The introduced traits in GTxLLxB2 are intended for agronomic benefits. Extensive compositional analysis was undertaken, taking into consideration the OECD consensus document on "compositional considerations for new varieties of cotton: key food and feed nutrients and anti-nutrients". No change in the nutritional composition was intended and upon extensive analysis, none was found.
The primary use of cotton is for the textile industry. However the by-products of cotton ginning find many uses in human and animal diets. Compositional equivalence was demonstrated for the food proprieties of the cottonseed oil. The key nutrients, fatty acids and vitamin E (tocopherol), which are the principal components of cottonseed oil, were investigated. The lipid profile is preserved in GTxLLxB2, and the fatty acid levels in the cottonseed oil samples are similar to those of the conventional cottonseed oil samples and within the range reported in the literature.
Cottonseed oil from GTxLLxB2 has the same nutritional composition as its conventional counterpart, and values for nutritional components fall within the range of values reported for cotton commodities in commerce.
In conclusion, vegetable oil derived from GTxLLxB2 cotton grain will be nutritionally equivalent to vegetable oil derived from commercially available cotton grain and there is no nutritional impact expected from the human food use of GTxLLxB2 cotton and derived food products.

7.10.2 Nutritional assessment of GM feed

Extensive compositional analysis was undertaken, taking into consideration the OECD consensus document on "compositional considerations for new varieties of cotton: key food and feed nutrients and anti-nutrients". The by-products of cottonseed processing (cottonseed meal and cottonseed hulls) can be used in animal feed. Cotton contains some anti-nutritional factors, most of which are concentrated in the meal fraction. The anti-nutritional compounds include gossypol and cyclopropenoid fatty acids, which are subject to heat denaturation. Cottonseed meal is typically subjected to a moist heat treatment to facilitate oil removal. This treatment denatures proteins and detoxifies the gossypol that otherwise would cause the cottonseed meal to be unsuitable as an animal feed. Anti-nutritional compounds common to cotton were best measured in toasted cottonseed meal and are well below acceptable levels, and similar to levels in conventional cotton.

Cottonseed meal and hulls derived from GTxLLxB2 grain will be nutritionally equivalent to cottonseed meal and hulls derived from commercially available cotton grain, and there is no nutritional impact expected from the animal feed use of GTxLLxB2 cotton and derived products.

7.11 Post-market monitoring of GM food/feed

No post-market monitoring plan is required for GM food/feed produced from GTxLL cotton

The near isogenic non GM FM958 variety was used in the comparative analysis of GTxLLxB2 (D.7.1, D.7.2 and D.7.3). The intent of the genetic modifications in the GTxLLxB2 combined event were for agronomic benefit (D.7.4), no change in the nutritional composition or value was intended (D.7.6, D.7.10). No health claims are intended. Food derived from GTxLLxB2 cotton will not be marketed as an alternative to or replacement for traditional cottonseed food products (D.7.5). GTxLLxB2 cotton has no specific properties that might increase the dietary intake compared to traditional cotton (D.7.7). There is no evidence that the long term nutritional and health status of some individuals of the European population could be impacted by the marketing of GTxLLxB2 cotton derived food products (D.7.8, D.7.9 and D.7.10) or of the subcombination LLxB2.

8. Mechanism of interaction between the GM plant and target organisms (if applicable)

GTxLLxB2 cotton differs from other cotton varieties by the tolerance to glyphosate and glufosinate-ammonium herbicides and resistance to key cotton insect pests of the lepidopteran family. These lepidopteran insects may be considered as targets organisms which interact with the GTxLLxB2 cotton plants.

However, the scope of this application is food and feed, import and processing of the GTxLLxB2 cotton and does not include cultivation in the EU. Therefore, no interactions between GTxLLxB2 cotton plants and lepidopteran insects are expected.

9. Potential changes in the interactions of the GM plant with the biotic environment resulting from the genetic modification

9.1 Persistence and invasiveness

an assessment of the agronomic performance of GTxLLxB2 cotton, including parameters related to reproductive and vegetative fitness was performed in field trial studies in USA during the 2008 growing season

In addition, the scope of this application is for authorization of GTxLLxB2 cotton for food and feed uses, as well as import and processing and does not include authorization for cultivation of GTxLLxB2 and the subcombination LLxB2 cotton seeds in the EU. As a consequence, exposure to the environment will be limited to unintended release of GTxLLxB2 and LLxB2, which could occur, for example, via accidental losses during transportation and processing

In conclusion, the likelihood for GTxLLxB2 cotton and the subcombination LLxB2 to become environmentally persistent or invasive giving rise to any weediness is negligible within the scope of this application.

9.2 Selective advantage or disadvantage

None. Agronomic performance shows no disadvantage in GTxLL xB2cotton. In very rare cases when such accidental spillage of cottonseed could occur and seeds would germinate, the GTxLLxB2 and LLxB2 plants would only have a selective advantage in the presence of glyphosate and/or glufosinate-ammonium herbicides and/or under severe lepidopteran pest infestation.
In conclusion, GTxLLxB2 cotton and the subcombination LLxB2 do not have any potential selective advantage or disadvantage in plant establishment in the European environment in comparison to other cotton, therefore no unintended effect on the environment is expected.

9.3 Potential for gene transfer

The scope of this application is for authorization of GTxLLxB2 cotton and the LLxB2 subcombination for food and feed uses, as well as import and processing and does not include authorization for cultivation of GTxLLxB2 and LLxB2 cotton seeds in the EU.

Plant to bacteria gene transfer

Since none of the genetic elements inserted in the GTxLLxB2 and LLxB2 cottons has a genetic transfer function, and no new genetic modification was introduced in these combined events, the possibility of transfer of genetic material from GTxLLxB2 and LLxB2 cottons to bacteria is equally unlikely as for their parents

Plant to plant gene transfer

The scope of this application does not include authorization for cultivation of GTxLLxB2 and LLxB2 cotton seeds in the EU. As a consequence, exposure to the environment will be limited to unintended release of GTxLLxB2 and LLxB2 cottons, which could occur through a seed spill as a result of import. Seeds that may escape during transport do not give rise to persistent populations due to the seed treatment requirements.

In conclusion, likelihood of unintended environmental effects as a consequence of gene transfer from GTxLLxB2 cotton or the subcombination LLxB2 is negligible.

9.4 Interactions between the GM plant and target organisms

The insect protection trait of GTxLLxB2 and of the subcombination LLxB2, inherited from MON 15985, provides control against certain key cotton pests of the lepidopteran family. However the scope of this application does not include cultivation of GTxLLxB2 or LLxB2 seeds in the EU and therefore exposure to the environment will be limited to unintended release of the GTxLLxB2 or LLxB2 cottonseed, which could occur via incidental spillage during transportation and processing. Taking into consideration the poor survival characteristics of conventional cotton under most European non-agricultural conditions and the fact that GTxLLxB2 and LLxB2 cotton will be imported as mostly non-viable seed, a likelihood that imported GTxLLxB2 and LLxB2 will establish a feral cotton population and will interact with potential target organisms affecting their insecticidal properties can be considered as negligible.

9.5 Interactions of the GM plant with non-target organisms

The intended uses of GTxLLxB2 and the subcombination LLxB2 specifically exclude cultivation, so the environmental exposure to GTxLLxB2 and LLxB2 is limited to the accidental release of grains into the environment during transportation and processing. It would need successful establishment and spread of high numbers of GTxLLxB2 and LLxB2 plants to enable any significant interaction with non-target organisms, which is very unlikely.

GTxLLxB2 and LLxB2 cottons were developed by the conventional crossing of GHB614, LLCotton25 and MON 15985 lines. Three possible interactions with other organisms were examined on the parental lines GHB614 and LLCotton25.

The genetic modification, tolerance to herbicide products containing glyphosate and glufosinate ammonium, did not change the interaction of GM cotton varieties with other organisms in the absence of herbicide application. Under agricultural conditions in the USA, when the herbicides are applied: i.) some advantage may be gained in plant population dynamics; ii.) in habitats outside agriculture, the interaction with other plant communities is similar to that of any other cotton; iii.) no changes could be identified in interactions with non-target organisms in the environments under which glyphosate and glufosinate ammonium tolerant cotton will be cultivated. Under agricultural conditions, with direct comparisons of herbicide application, insect population diversity and measures of sensitivity to natural pathogens of cotton found no advantage for the transgenic events GHB614 and

LLCotton25.

a) Effects on biodiversity in the area of cultivation

Under selection pressure within the area treated with herbicide products, containing glyphosate or glufosinate ammonium, GHB614 and LLCotton25 may establish in the environment and, thereby, modify the biodiversity.
The introduced lepidopteran-protection trait confers a selective advantage only under specific conditions (i.e. upon attack be target insects), which are short in duration. The advantage is of purely agronomic interest and presents negligible risk to the non-agricultural environments, and because of the poor survival of cotton under most European conditions.
Furthermore it might transfer the trait via pollen flow to other cultivated cotton (wild relatives of cotton are not found in Europe) in the vicinity.

b) Effects on biodiversity in other habitats

GTxLLxB2 and LLxB2 will be imported primarily as non-viable seed. Therefore the likelihood that some imported seed could escape from silos or lorries and germinate is very low. In the unlikely event that GTxLLxB2 and LLxB2 plants would germinate, they would only have a selective advantage in those cases where herbicide products containing glufosinate ammonium and/or glyphosate are used. In all other cases, the likelihood to establish a feral population of GTxLLxB2 and LLxBB2 is no higher than for conventional cotton.

c) Effects on non-target organisms

There are no non-target organisms specific to GHB614 and LLCotton25. All non-target organisms would be the same as for conventional cotton. There are no observed effects of the herbicide-tolerant cotton on non-target organisms. Under agricultural conditions, with direct comparisons of glyphosate/glufosinate herbicide application, insect population diversity and measures of sensitivity to natural pathogens of cotton found no advantage for events GHB614 and LLCotton25. Field observations found no differences in insect populations, or reactions to natural infestation of cotton pathogens.

In relation to the insect resistance trait, the more targeted application of the insecticidal chemical (plant expression as opposed to spray application) may limit the exposure level of many field arthropods. Beneficial, non-target arthropods can become exposed to insecticidal proteins produced in transgenic plants in several ways: by feeding on the plant parts themselves or through feeding on target or non-target herbivorous insects. Studies on the effect of *B.t.* crops on non-target arthropods found no significant effect on abundance of generalist predators

Considering the scope of the application (that excludes cultivation) and the intended uses of GTxLLxB2 and LLxB2 cottons, it can be concluded that the exposure of potentially sensitive non-target organisms to the Cry1Ac and Cry2Ab2 proteins is likely to be very low and of no ecological relevance, especially as it has been demonstrated that the insecticidal proteins inherited from MON 15985 are very specific of the target lepidopteran pests, and are safe to non-target organisms.

Therefore; the likelihood for direct or indirect interactions between GTxLLxB2 or LLxB2 and non-target organisms in the environment is considered negligible

9.6 Effects on human health

GTxLLxB2 cotton has been obtained by conventional crossing between the parental lines GHB614, LLCotton25 and MON 15985. No new genetic modification was used for the development of GTxLLxB2 cotton.

All three parental lines of GTxLLxB2 have already been notified and submitted to a risk assessment. In regard to LLCotton25 and GHB614 cotton, the EFSA GMO Panel concluded that LLCotton25 and GHB614 events are as safe and as nutritious as conventional cotton for humans and animals. MON15985 is currently under EFSA risk assessment.

In the current application, it has been demonstrated that there are no interactions between GHB614, LLCotton25 and MON 15985 events when combined by means of a conventional breeding cross.

In conclusion, this confirms that GTxLLxB2 cotton is as safe as and as nutritious as any commercial cotton.

9.7 Effects on animal health

GTxLLxB2 cotton has been obtained by conventional crossing between the parental lines GHB614, LLCotton25 and MON 15985. No new genetic modification was used for the development of GTxLLxB2 cotton.

All three parental lines of GTxLLxB2 have already been notified and submitted to a risk assessment. In regard to LLCotton25 and GHB614 cotton, the EFSA GMO Panel concluded that LLCotton25 and GHB614 events are as safe and as nutritious as conventional cotton for humans and animals. MON15985 is currently under EFSA risk assessment.

In this application no differences were identified for nutritive value of the seed and no indications of toxic or adverse effects were associated with any of the sources of cotton in the tested animal species.

In conclusion, cottonseed of the combined events cotton GTxLLxB2 and the subcombination LLxB2 is as safe as and as nutritious as any commercial cotton..

9.8 Effects on biogeochemical processes

As described in Section D.7.4, an assessment of the agronomic performance of GTxLLxB2 cotton was performed in field trial studies in USA during the 2008 growing season. No differences were observed in agronomic studies that would indicate a negative effect on biogeochemical processes resulting from the cultivation of GTxLLxB2.

In conclusion, negligible effects are expected on the biogeochemical processes occurring in the soil within the context of the current application.

9.9 Impacts of the specific cultivation, management and harvesting techniques

Not applicable since the scope of this application is authorization of GTxLLxB2 and LLxB2 cottons for food and feed uses, and import and processing and does not include authorization for cultivation of GTxLLxB2 or LLxB2 cottonseed in the EU.

10. Potential interactions with the abiotic environment

GTxLLxB2 cotton and the subcombination LLxB2 combine the tolerance to glufosinate-ammonium and/or glyphosate herbicides with an insect protection trait. The traits in GTxLLxB2 and LLxB2 cotton are not aimed at modifying the interactions of the plant with the abiotic environment.

As presented in Section D.7.3, chemical analysis of the nutritional components in GTxLLxB2 cottonseed found no differences in the mineral composition and thus no reason to consider mineral utilization from the soil to be different than for commercially available cotton.

Furthermore, the scope of this application is for authorization of GTxLLxB2 and LLxB2 cottons for food and feed uses, and import and processing, and does not include authorization for cultivation of GTxLLxB2 or LLxB2 seeds in the EU.

11. Environmental monitoring plan

11.1 General (risk assessment, background information)

As required by Article 5(5)(b) and 17(5)(b) of Regulation (EC) No. 1829/2003 the proposed monitoring plan for GTxLLxB2 cotton has been developed according to the principles and objectives outlined in Annex VII of Directive 2001/18/EC and Decision 2002/811/EC establishing guidance notes supplementing Annex VII to Directive 2001/18/EC. The structure of the monitoring plan also takes into account the guidance on presentation of applications provided in the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (The EFSA Journal (2006) 99, pp. 1-100).

11.2 Interplay between environmental risk assessment and monitoring

The scope of this application is the authorisation of GTxLLxB2 cotton varieties and the subcombination LLxB2 for import, processing, food and feed use in the European Union (EU) under Regulation (EC) No. 1829/2003. The scope of the application does not include authorisation for the cultivation of GTxLLxB2 cotton seed products or the cultivation of the subcombination LLxB2 in the EU.

An environmental risk assessment (e.r.a.) was carried out for GTxLLxB2 cotton and for the subcombination LLxB2 according to the principles laid down in Annex II to Directive 2001/18/EC and Decision 2002/623/EC establishing guidance notes supplementing Annex II to Directive 2001/18/EC. The scientific evaluation of the characteristics of GTxLLxB2 cotton in the e.r.a. has shown that the risk for potential adverse effects on human and animal health or the environment is negligible in the context of the intended uses of GTxLLxB2 and LLxB2 cotton

11.3 Case-specific GM plant monitoring (approach, strategy, method and analysis)

The scientific evaluation of the characteristics of GTxLLxB2 cotton and the subcombination LLxB2 in the e.r.a. has shown that the risk for potential adverse effects on human and animal health or the environment is negligible in the context of the intended uses of GTxLLxB2 cotton. It is therefore considered that there is no need for a case-specific monitoring.

11.4 General surveillance of the impact of the GM plant (approach, strategy, method and analysis)

The objective of general surveillance is to identify the occurrence of unanticipated adverse effects of the viable GMO or its use on human or animal health or the environment that were not predicted in the e.r.a.

The baseline and controls for general surveillance will rely on the historical knowledge and experience with non-GM cotton as comparable reference where necessary as the intended uses are the same as that of any other commercial cotton. The people and their networks participating in the surveillance plan, such as operators involved in the import, handling and processing of viable GTxLLxB2 cotton, would tend, although not exclusively, to be best suited to observe and report any unanticipated adverse effect in the framework of their routine surveillance of the commodities they handle and use. They will report immediately any adverse effect to Bayer CropScience, who will directly investigate and inform the European Commission in accordance with Regulation (EC) No 1829/2003, or at least annually whether or not a potential adverse effect was observed.

The operators will be provided with guidance to facilitate reporting of any unanticipated adverse effect from handling and use of viable GTxLLxB2 cotton. Bayer CropScience will provide appropriate technical information on GTxLLxB2 and further information on the product and relevant legislation will be available from a number of sources, including industry and government websites, official registers and government publications.

The general surveillance information reported to and collected by Bayer CropScience from the European trade associations or other sources will be analyzed for its relevance. Where information indicates the possibility of an unanticipated adverse effect, Bayer CropScience will immediately investigate to determine and confirm whether a significant correlation between the effect and GTxLLxB2 cotton can be established.

11.5 Reporting the results of monitoring

In accordance with Regulation (EC) No 1829/2003, the authorisation holders are responsible to inform the European Commission of the results of the general surveillance.

If information that confirms an adverse effect of GTxLLxB2 or LLxB2 cotton and that alters the existing risk assessment becomes available, the authorisation holders will immediately investigate and inform the European Commission. The authorisation holders, in collaboration with the European Commission and based on a scientific evaluation of the potential consequences of the observed adverse effect, will define and implement management measures to protect human and animal health or the environment, as necessary. It is important that the remedial action is proportionate to the significance of the observed effect.

The authorisation holders will submit an annual monitoring report including results of the general surveillance in accordance with the conditions of the authorisation. The report will contain information on any unanticipated adverse effects that have arisen from handling and use of viable GTxLLxB2 or LLxB2 cotton.

The report will include a scientific evaluation of the confirmed adverse effect, a conclusion of the safety of GTxLLxB2 and LLxB2 cotton and, as appropriate, the measures that were taken to ensure the safety of human and animal health or the environment.

The report will also clearly state which parts of the provided information are considered to be confidential, together with a verifiable justification for confidentiality in accordance with Article 30.

12. Detection and event-specific identification techniques for the GM plant

The detection method for GTxLLxB2 cotton is based on the validated detection methods that are available for GHB614, LLCotton25 and MON 15985cotton.

The detection method for GTxLLxB2 cotton has been sent to the Community Reference Laboratory (CRL) (http://gmo-crl.jrc.ec.europa.eu/statusofdoss.htm) of the Joint Research Centre of the European Commission (EC-JRC) for the purpose of experimental testing and validation.

Appropriate control samples have also been made available to the JRC-CRL.

E. INFORMATION RELATING TO PREVIOUS RELEASES OF THE GM PLANT AND/OR DERIVED PRODUCTS

1. History of previous releases of the GM plant notified under Part B of the Directive 2001/18/EC and under Part B of Directive 90/220/EEC by the same notifier

a) Notification number

There is no history of release of GTxLLxB2 cotton in the EU.

b) Conclusions of post-release monitoring

Not applicable.

c) Results of the release in respect to any risk to human health and the environment (submitted to the Competent Authority according to Article 10 of Directive 2001/18/EC)

Not applicable.

2. History of previous releases of the GM plant carried out outside the Community by the same notifier

a) Release country : GTxLLxB2 has been field tested in the USA since 2008 (permit number 08-119-101n) and in South Africa in 2009 (permit number 17/3(4/09/024). Field tests of LLxB2 in the US and Australia have been conducted since 2002, and in South-Africa in 2008 (permit numbers 17/3(2/08/233); 17/3(4/08/234)) and 2009 (permit numbers 17/3(2/09/187); 17/3(4/09/188)).
b) Authority overseeing the release USA: United States Department of Agriculture (USDA). South-Africa: Biosafety Directorate, Department of Agriculture, Forestry & Fisheries Australia: Office of Gene Technology Regulator.
c) Release site USA: United States Department of Agriculture (USDA). South-Africa: Biosafety Directorate, Department of Agriculture, Forestry & Fisheries Australia: Office of Gene Technology Regulator.
d) Aim of the release See E.2.a., field releases for breeding and variety development, technical developments for best agronomic practices and cotton integrated pest management systems have been conducted.
e) Duration of the release The generation time for cotton from planting to harvest is 100 to 200 days.
f) Aim of post-releases monitoring Volunteer GTxLLxB2 plants in subsequent season.
g) Duration of post-releases monitoring One or two seasons, until no volunteers observed
h) Conclusions of post-release monitoring Occurrence of volunteers is very infrequent and dependent upon mild conditions in the winter season.
i) Results of the release in respect to any risk to human health and the environment No risk to human health or the environment has been indicated by the field release experience.

3. Links (some of these links may be accessible only to the competent authorities of the Member States, to the Commission and to EFSA):

a) Status/process of approval The JRC websites http://gmoinfo.jrc.ec.europa.eu/gmp_browse.aspx and http://gmo-crl.jrc.ec.europa.eu/statusofdoss.htm provide publicly accessible links to up-to-date databases on the regulatory progress of notifications under Directive 2001/18/EC and Regulation (EC) No 1829/2003.
b) Assessment Report of the Competent Authority (Directive 2001/18/EC) A notification for GTxLLxB2 cotton according to Directive 2001/18/EC has not been submitted by Bayer CropScience.
c) EFSA opinion Not available at the time of submission of this application.
d) Commission Register (Commission Decision 2004/204/EC) Not available at the time of submission of this application.
e) Molecular Register of the Community Reference Laboratory/Joint Research Centre Information on detection protocols will likely be posted at http://gmo-crl.jrc.it/statusofdoss.htm.
f) Biosafety Clearing-House (Council Decision 2002/628/EC) http://bch.biodiv.org/
g) Summary Notification Information Format (SNIF) (Council Decision 2002/812/EC) http://gmoinfo.jrc.ec.europa.eu/