Notification

Glufosinate tolerant Cotton
Transformation event LLCotton25

Summary Information Format

March 2004

SUMMARY INFORMATION FORMAT FOR PRODUCTS CONTAINING GENETICALLY MODIFIED HIGHER PLANTS (GMHPs)

A. GENERAL INFORMATION

1. Details of notification

a) Member State of notification:	**Spain**
b) Notification number:	**C/ES/04/02**
c) Name of the product (commercial and other names): Cotton seeds, and derived products, of GM cotton (*Gossypium hirsutum*) with tolerance to glufosinate-ammonium derived by classical breeding methods from crosses between GM cotton transformation event LLCotton25 (UIC: OECD ID: ACS-GHØØ1-3) and non-GM cotton cultivars.	
d) Date of acknowledgement of notification:	

2. Notifier

a) Name of notifier: Bayer CropScience GmbH
b) Address of notifier: Industriepark Hoechst, K607, Brüningstrasse 50, D-65926 Frankfurt am Main, Germany
c) Is the notifier: domestic manufacturer: No ☐ importer: Yes ☒
d) In the case of an import the name and address of the manufacturer shall be given Bayer CropScience L.P. 2 T.W. Alexander Drive Research Triangle Park NC 27709 USA.

3. General description of the product

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

The recipient is a commercial variety of cotton, Coker 312.
The genetic modification aims to develop cottons, tolerant for glufosinate-ammonium (GA) herbicides.

b) Any specific form in which the product must not be placed on the market (seeds, cut-flowers, vegetative parts, etc.) as a proposed condition of the authorisation applied for

None.

c) Intended use of the product and types of users

Cottonseed products derived from event LLCotton25 will be imported in the EU from the major cotton growing areas as commodity and could be used for downstream purposes as food, feed and industrial products identically to non-GM cottons.

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

Cotton seeds deriving from the event LLcotton25 can be used, stored and handled in a similar way as other cottons presently on the market.

If GM cotton is co-mingled with non-genetically modified cotton during use, storage and handling, the corresponding batch will have to be labelled according to the legislation in application to the EU.

e) If applicable, geographical areas within the EU to which the product is intended to be confined under the terms of the authorisation applied for

Not applicable.

f) Any type of environment to which the product is unsuited

None

g) Any proposed packaging requirements

There is no reason for specific packaging requirements.

h) Any proposed labelling requirements in addition to those required by law

Imported crop will enter the EU as a commingled commodity along with other cotton seed from the major cotton growing areas. The shipments are intended to bear no label but the accompanying compulsory documents indicating “contains Genetically Modified Cotton” will be delivered, together with the unique identification code.
The language of the label will be adapted according to legal requirements of the Cartagena Protocol on Biosafety to the Convention on Biological Diversity and the regulation (proposal 2001/018/COD of the European Community concerning traceability of food and feed products produced from GMOs and amending Directive 2001/18/EC), as these initiatives come into force.
Additionally, all necessary information relating to the genetically modified cotton subject to this notification produced by or under licence from Bayer CropScience GmbH outside the EU will be delivered to those companies which are known to import the crop into the EU for processing.

i) Estimated potential demand

(i) in the Community: Between 1996 and 2000 the EU imported yearly cotton derived commodities such as lint, cottonseed cake and meal, cottonseed, cotton waste, linters, cottonseed oil, carded combed cotton in an average volume of 0.78, 0.19, 0.19, 0.14, 0.08, 0.01 and 0.003 million tons respectively, meaning a combined value of approximately 200 million Euro. The EU's imported cotton originated partly in US, Central Asia and in West Africa. The largest cotton importers in the period analysed were Italy, Spain and Germany.

LLCotton25 derived products could reach as much as 10 % of the total Community demand.

(ii) in export markets for EC supplies: Not relevant.

j) Unique identification code(s) of the GMO(s)

Transformation event LLCotton25 : **OECD ID: ACS-GHØØ1-3**

4. Has the GMHP referred to in this product 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

Risk assessment information provided in following points 9-11, 14-27, 29-33.

5. Is the product being simultaneously notified to another Member State ?

Yes ☐	No ☒

If no, refer to risk analysis data on the basis of the elements of Part B of Directive 2001/18/EC

Risk assessment information provided in following points 9-11, 14-27, 29-33.

Or

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

Yes ☒	No ☐

If yes, please specify:

USA (commercial uses)
Canada, Mexico, Japan, Australia (import)

6. Has the same GMHP been previously notified for marketing in the Community?

Yes ☐	No ☒
If yes, give notification number and Member State	

7. Measures suggested by the notifier 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 products from different levels of downstream processing (point 3.i) without the ability for natural reproduction. Viable cottonseed will be imported in small portions only. The safety profile in terms of human and animal health and environmental impact of seeds of LLCotton25 and conventional cottons are identical and do not constitute hazard.

In the case of accidental spillage of LLcotton25 in transit or at the processing facility, the area will be monitored for one season for the germination and plant establishment of the spilled cottonseed.

B. NATURE OF THE GMHP CONTAINED IN THE PRODUCT

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

8. Complete name

a) Family name:	Malvaceae
b) Genus:	*Gossypium*
c*) Species*:	*hirsutum*
d) Subspecies:	Not applicable
e) Cultivar/breeding line:	Coker312
f) Common name:	cotton

9. a) Information concerning reproduction

(i) Mode(s) of reproduction

Vegetative proliferation of cotton requires human intervention; therefore its mode of reproduction can be restricted to **sexual reproduction** only.

Cotton is mainly an **autogamous** species, however some degree of insect mediated **cross-pollination** may take place.

Out-crossing rates in the range of 1 - 28% have been observed in *G. hirsutum* to other *G. hirsutum* cultivars in adjacent plots, which varied with locations and years, but declined rapidly with distance. Findings carried on in Spain have shown very low rates of natural crossing in normal crop conditions.

Further evidence for the limited amount of cross-pollination that occurs in cotton comes from the limited isolation distance required (30m) established for certification of hybrid plant materials by AOSCA Handbook.

(ii) Specific factors affecting reproduction, if any

Main abiotic environmental factors affecting cotton reproduction that determine the cotton production areas too are **temperature profile**, such as 1.) active vegetative growth range: 15 - 38 °C, 2.) accumulated heat GD15.5°C need: 1,200 unit, 3.) number of frost free days: 200, 4.) rapid and consistent spring warming pattern, as well as high **light intensity.**
The **frequency of cross-pollination** varies with **the insect pollinator population** in particular with various wild bees, bumble bees (*Bombus* ssp.) and honey bees (*Apis mellifera*). 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.

(iii) Generation time

Cotton in nature is a perennial shrub, which has been domesticated and converted to an annual crop. The generation time of cultivated cotton varies between 100 and 200 days.

9. *b) Sexual compatibility with other cultivated or wild plant species*

There are no identified non *G. hirsutum* plants that are sexually compatible with cultivated cotton in the EU.

Pre-zygotic, and ***post-zygotic*** **barriers** greatly limit the sexual compatibility of *G. hirsutum* with other plant species in the Gossypiae tribe. In addition plants of the *Gossypium* genus are not native to Europe. Several members of the genus 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 sp*. failed or resulted in sterile seeeds.

G. hirsutum, an allotetraploid species that combines the AADD genomes, will hybridise only with other tetraploid members of the *Gossypium* genus including *G. tomentosum, G. darwinii , G. mustelinum, G. hirsutum – G. lanceolatum, and G. barbadense*, which species are not known to have a habitat in Europe.

10. Survivability

a) Ability to form structures for survival or dormancy

Cotton is a perennial plant that is harvested and planted annually. Cultivated cotton reproduces sexually, therefore seeds are the only structure for survival. Some wild forms may produce "hard seeds" that, upon drying, become impermeable to water and suffer delayed germination. Hard seeds however are undesirable agronomically and the trait has been largely eliminated from modern commercial cultivars through breeding and selection.

Cultivated cotton is not considered to produce seeds, which can persist in the environment for long period of time, and cottonseed lacks the ability to develop dormancy.

b) Specific factors affecting survivability, if any

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, it is likely to rot.

In most cotton growing areas of Europe some of the seed remaining in the field following harvest and soil cultivation may germinate in the autumn if the conditions are favourable. Seeds not germinating are beginning to rot and die. In cotton growing regions with mild and dry winters, such as Spain and Greece, cotton 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.

11. Dissemination

a) Ways and extent of dissemination

The two differentiated structures suitable for dispersal of cotton genes in the environment are the seed and pollen.
Seed dispersal could occur during transport, at sowing and essentially before and during harvest.
Pollen dispersal studies conclude that when out-crossing occurs, it is localised around the pollen source and decreases significantly with distance. Pollen movement was traced by means of fluorescent particles and found that even among flowers located only 50 to 60 m from a cotton field, surrounded by a large number of bee colonies, fluorescent particles were detected on only 1.6 % of the flowers.

b) Specific factors affecting dissemination, if any

Seed dispersal: Cotton seed has no structural modifications to facilitate transfer by animals. Dissemination is mainly the result of human activity.

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

12. Geographical distribution of the plant

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.

G. hirsutum, known as upland or Mexican cotton represents over 90 % of world-wide production besides one only "New Word" tetraploid species: *G. barbadense* (known as Pima or South American cotton) and two "Old Word" diploid species: *G. arboreum* and *G. herbaceum*. Main cotton producers are China, USA, India, Pakistan, Uzbekistan, Brazil and Turkey.

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. All grow around beaches or are confined on small islands.

13. 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

Cotton is commercially grown in **Spain** and **Greece**.

14. Potentially significant interactions of the plant with other organisms in the ecosystem where it is usually grown, 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, surrounding weed species, animals and humans**. The crop has been cultivated in Spain and Greece for decades and has a history of safe use.

Cotton crop during thousands of years was produced for fibre, in the XXth century it turned to food/feed channels. Cotton is not considered harmful or pathogenic to animals or humans, however the plant does produce a small amount of natural antinutritional factors such as **gossypol, cyclopropenoid fatty acids** and **phytic acid.**

With the exception of phytic acid, all the antinutritionals are subjected to neutralisation during processing. Free gossypol bounds to lysine, which is then unavailable to animals. Cyclopropenoid fatty acids are deactivated or removed from the oil by hydrogenation or during deodorization at 230-235°C.

15. Phenotypic and genetic traits

No specific genetic markers relevant to this request are present in the parental line.

The recipient variety Coker312 (PVP 7200100) is an U.S. Protected, glanded Variety of SEEDCO Corporation, Texas.

INFORMATION RELATING TO THE GENETIC MODIFICATION

16. Description of the methods used for the genetic modification

The genetic modification was performed by *Agrobacterium* mediated transformation using a binary vector system containing the chimeric gene denoted as P*35S-bar*-3'*nos*.

17. Nature and source of the vector used

Plasmid pGSV71 is a derivative of pGSV1, which was constructed in *Escherichia coli*, and thereafter transferred to a suitable *Agrobacterium tumefaciens* strain.

18. Size, source [name of donor organism(s)] and intended function of each constituent fragment of the region intended for insertion

Table 1. Size, source and intended function of each constituent fragment of the region intended for insertion

Nt position	Source	Size (bp)	Reference	Intended function
0198 – 0222	Right border repeat from the TL-DNA from pTiB6S3	24	Gielen *et al*., 1984	None; remaining part of the vector
0223 – 0249	Synthetic	26		Polylinker sequence
0250 – 1634	**P*35S***: promoter region from the Cauliflower Mosaic Virus	1384	Odell *et al*., 1985	Regulatory sequence for high level constitutive expression in the plant
1635 – 2186	***bar***: the coding sequence of the bialaphos resistance gene of *Streptomyces hygroscopicus.*	551	Thompson *et al*., 1987	Herbicide tolerance and selectable marker
2187 – 2205	Synthetic	18		Polylinker sequence
2206 – 2465	**3'*nos***: a *Taq*I fragment from the 3' untranslated end of the nopaline synthase gene from the T-DNA of pTiT37	259	Depicker *et al*., 1982	Polyadenylation signal
2466 – 2519	Synthetic	53		Polylinker sequence
2520 – 2544	Left border repeat from the TL-DNA from pTiB6S3	2	Gielen *et al*., 1984	None; remaining part of the vector

Depicker A., Stachel S., Dhaese P., Zambryski P., Goodman H.M. 1982. Nopaline synthase: transcript mapping and DNA sequence. Journal of Molecular and Applied Genetics, 1, 561-573.

Gielen J., De Beuckeleer M., Seurinck J., Deboeck F., De Greve H., Lemmers M., Van Montagu M., Schell J. 1984. The complete nucleotide sequence of the TL-DNA of the *Agrobacterium tumefaciens* plasmid pTiAch5. The EMBO Journal 3, 835-846.

Odell J.T., Nagy F., Chua N.-H. 1985. Identification of DNA sequences required for activity of the Cauliflower Mosaic Virus 35S promoter. Nature 313, 810-812.

Thompson C.J., Rao Movva N., Tizard R., Crameri R., Davies J., Lauwereys M., Botterman J. 1987. Characterization of the herbicide resistance gene bar from *Streptomyces hygroscopicus*. The EMBO Journal 6, 2519-2523.

INFORMATION RELATING TO THE GMHP

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

Herbicide tolerance
Tolerance is based upon the *bar* gene, a glufosinate resistance gene, isolated from the soil micro-organism, *Streptomyces hygroscopicus*. Using recombinant DNA technologies, the *bar* gene has been cloned from *S. hygroscopicus*, fused with the *35S* promoter from Cauliflower Mosaic Virus and introduced into the plant genome. The *bar* gene, when expressed, enables the production of the enzyme, Phosphinothricin-Acetyl-Transferase (PAT) that acetylates L-glufosinate and thereby conferring tolerance to herbicides based upon glufosinate ammonium.

20. Information on the sequences actually inserted/deleted/modified

a) Size and structure of the insert and methods used for its characterisation, including information on any parts of the vector introduced in the GMHP or any carrier or foreign DNA remaining in the GMHP

The inserted DNA sequence has a length of 2319 bp.

Southern blot hybridization data with genomic DNA cut with five different restriction enzymes demonstrate that the event LLCotton25 contains **one intact copy of the gene cassette**. Transformation event LLCotton25 **contains no vector backbone** sequences as evidenced by using overlapping probes which cover the complete pGSV71 vector backbone sequences (including *Sm/Sp*, pVS1*ori* and *ColE1*).

PCR analysis demonstrates that the complete Right Border repeat is not inserted into transformation event LLCotton25 and that the endpoint of the T-DNA is located within the Left Border direct repeat.

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

Not applicable.

c) Location of the insert in the plant cells (integrated in the chromosome, chloroplast, mitochondrion, or maintained in a non-integrated form), and methods for its determination

The insert is integrated in a single genetic locus in the cotton nuclear genome (chromosome) as demonstrated by Mendelian inheritance patterns, Southern hybridisation probing of the genomic DNA and analysis of the flanking regions.

There is no indication that insertion of the DNA has a negative or unintended effect.

d) Copy number and genetic stability of the insert

Southern analysis and sequencing of the insert shows that LLcotton25 contains one *bar* gene cassette.

Mendelian inheritance patterns and Southern blots demonstrate the molecular stability of the cotton event LLcotton25 over several generations and under different genetic backgrounds and locations.

e) In case of modifications other than insertion or deletion, describe function of the modified genetic material before and after the modification as well as direct changes in expression of genes as a result of the modification

Not applicable.

21. Information on the expression of the insert

a) Information on the expression of the insert and methods used for its characterisation

Linked to the plant promoter, *35S*, the expression of the *bar* gene is targeted to green tissue of the plant. Tissue samples were harvested from greenhouse-grown cotton at the 2-4 leaf development stage. The expression level was measured by PAT protein specific ELISA.

It was found that PAT protein constituted 8 µg/g fresh weight of roots, 37 µg/g fresh weight of stems and 53 µg/g fresh weight of leaves. PAT protein comprises an average of 0.08, 0.23 and 0.19% of the total crude protein in roots, stems and leaves respectively, of cotton event LLCotton25. The limit of detection of the assay for the different matrices was 6.4 ng/g for roots, 14.7 ng/g for stems and 8.0 ng/g for leaves.

The average PAT protein content of fresh pollen from individual flowers was 19.2 ug/g, which was about 2.3 times higher than for the frozen pollen sample. The PAT content as % of crude protein was not determined for pollen because the amount of material was much less than the minimum amount required for the analysis.

b) Parts of the plant where the insert is expressed (e.g. roots, stem, pollen, etc.)

From published experience with the 35S promoter in cotton, high level of expression of PAT protein was expected in the leaves and lesser amounts in other organs. Indeed the following order of PAT expression was found in the various tissues: seed >> leaf >>> stem >> fresh pollen >> roots, frozen pollen. For estimate of exposure, the amount of PAT protein in the leaves of LLCotton25 has an upper limit of approximately 130 µg/g fresh weight. The amount of PAT protein in seed is 70 µg/g fresh weight.

22. Information on how the GMHP differs from the recipient plant in

a) Mode(s) and/or rate of reproduction

Reproductive characteristics and agronomic parameters which may relate to any fitness advantages of LLcotton25 were found to be identical to its non-transgenic counterpart Coker312.

b) Dissemination

The genetic modification has not changed any of the seed and pollen dispersal patterns of LLcotton25. In **seed** dispersal of cotton the post – harvest human intervention may play a significant role. The introduced herbicide tolerance is an agronomic trait that can modify the herbicide use practice in cotton field only, but will not change post-harvest operations. Since seeds from the transgenic line and its counterpart share the same morphology, weight and size, there is no reason to suppose any increased seed dispersal by animal transportation.
Pollen- and flower morphology, pollen germination and pollen viability comparison studies showed no differences between LLcotton25 and Coker312. The genetic modification does not target the existing pre- and post-zygotic barriers of sexual compatibility of cotton, therefore pollen dispersal of LLcotton25 in Europe remains limited only to other New World cotton cultivators. Pollen dispersal experiments of LLcotton25 show an out-crossing rate similar to that found with the non-transgenic counterpart.

c) Survivability

Seed characteristics that impact survival and contribute in acquiring a weedy behaviour such as number of seeds per boll, number of seeds per plant, seed index, seedling vigor, stand counts (germination under field conditions) were compared in LLCotton25 and the isogenic non-transgenic lines. In all cases, no differences were observed in any of the seed characteristics.

d) Other differences

The only difference is that the GMHP survives applications with a herbicide based on the active ingredient glufosinate- ammonium.

23. Potential for transfer of genetic material from the GMHP to other organisms

a. Transfer of genetic material to other higher plants

Vertical gene transfer (to cultivated cotton varieties or wild relatives only): There is no evidence of genetic transfer and exchange under natural conditions with organisms other than those with which cotton is able to produce fertile crosses through sexual reproduction. The genetic modification does not change the overall genetic characteristics of the event and the genetic compatibility to related species for hybridisation remains unchanged. In the absence of endemic wild species, vertical gene transfer in Europe is restricted to cultivated cottons only.

Likelihood of gene flow: Gene flow can occur into an adjacent cotton crop, however the rate is likely to be very low because there exists a combination of botanical, geographic and agricultural barriers to gene flow. Findings from the environmental risk assessment of LLCotton25 support the following conclusions:

Measurement of outcrossing frequency

- Standards for cotton breeding and seed production in the USA are based upon isolation distances of 30 meters.
- Experience with glufosinate-tolerant cotton does not show an increase in outcrossing frequency.

Barriers to gene flow

- Gene flow via pollen and no other mechanism;
- Crossing is possible within the AADD genome, however no wild cottons exist in the EU;
- *G. hirsutum* is mostly a self-pollinating crop;
- No insect pollen vectors that could move the pollen long distances.

Consequence of gene flow
The transfer of the *bar* gene into other cultivated cotton will not adversely impact agriculture.

b. Transfer of genetic material to bacteria

In order for any horizontal gene transfer to lead to a new type of micro-organism and therefore to introduce a significant impact, some of the following conditions will have to be fulfilled:

- the uptake should result in the incorporation of complete undegraded DNA;
- the plant targeted genes should result in significant expression in a prokaryotic background;
- the expression should represent a significant increase over the background level;
- the traits should convey a competitive advantage to the strain in which they are incorporated.

In the very unlikely case where both horizontal gene transfer from genetically modified plants to bacteria would occur and where due to genetic recombination the genes would be expressed in micro-organisms (the *bar* gene is under the control of the *35S* promoter, which is not functional in bacteria), this would have no impact since the transgene would not provide a selective advantage (the substrate for PAT protein is the herbicide glufosinate ammonium).

Furthermore, the *bar* gene present in cotton event LLCotton25 was isolated from a naturally occurring soil microbe.

24. Information on any harmful effects on human health and the environment, arising from the genetic modification

Genetic transformation in LLcotton25 did not result in any harmful effects on human health or on the environment.
The only change present in the composition of LLCotton25 is the *bar* gene product **phosphinotricin-N- acetyl-transferase** (PAT) protein.
The PAT protein is not toxic for mammals and does not possess any of the characteristics associated with food allergens. Findings to support this conclusion include:

(i) The coding sequence of the *bar* gene is derived from a common soil microbe not known to be a pathogen.
(ii) The PAT protein has no homology with any known allergens, toxins and anti-nutrients
(iii) The PAT protein has no glycolysation sites present on certain food allergens.
(iv) The PAT protein is a heat labile and acid labile protein unlike most food allergens.
(v) The PAT protein is readily degraded and denatured by enzymes and acid present in gastric fluids of domestic animals and man.
(vi) The PAT protein is highly substrate specific. It acts on its target, glufosinate, but it does not act on glutamate, the closest structural analogue of L-glufosinate.

Compositional studies on raw agricultural commodities and processed cotton fractions including the proximates, fatty acid analysis, amino acid content, vitamins, minerals, as well as known anti-nutrients, demonstrate that LLcotton25 is no different from non-transgenic cotton with the exception of the expressed PAT protein.
During field tests of LLcotton25 no toxicity or alteration of population levels has been observed for beneficial insects, birds or other species that frequent cotton fields. There were no qualitative differences between beneficial species and populations present on transgenic and non-transgenic cotton plants.

25. Information on the safety of the GMHP to animal health, where the GMHP is intended to be used in animal feedstuffs, if different from that of the recipient/parental organism(s)

There is no difference between LLcotton25 and the recipient Coker 312 in terms of safety to animals. Cottonseed and the by-products of cotton processing are often included in animal diets. The nutritional composition of the seed and processed fractions (hulls, delinted seeds, meal, toasted meal) were found to be equivalent to other cotton by chemical analysis.
To support the finding of nutritional equivalence, poultry and dairy cows were fed diets containing cotton under conditions designed to evaluate growth and/or health parameters. Poultry were selected to evaluate the effects of a feed component over an approximately 5-week period and under conditions of very rapid growth, thus the assay is highly sensitive for nutritional or toxic effects. As the presence of gossypol greatly limits the use of cottonseed or cottonseed meal in animals other than ruminants, milk production parameters in dairy cows are a sensitive indicator of body condition. 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 either of the tested animal species.
Data and findings in every case lead to the conclusion that LLcotton25 is substantially equivalent to other varieties of cotton and there is no concern for the safety and nutrition of transformation event LLcotton25 and its progeny.

26. Mechanism of interaction between the GMHP and target organisms (if applicable) if different from that of the recipient/parental organism(s)

Not applicable as there are no target organisms.

27. Potentially significant interactions with non-target organisms, if different from the recipient or parental organism(s)

There are no non-target organisms specific to the GMHP compared to non-genetically modified cotton.
Mammals and other species which consume vegetation avoid feeding on that crop due to both gossypol content of the vegetative tissues and boll morphology. Seeds are within the boll and covered with lint. Therefore, birds, mammals and other wildlife are not expected to be significantly exposed in nature.

28. Description of detection and identification techniques for the GMHP, to distinguish it from the recipient or parental organism(s)

Phenotypic detection techniques based on the gufosinate tolerance of LLcotton25 allow an easy detection by a simple spraying with glufosinate herbides.

DNA-based methods:

- Discriminating PCR: A PCR protocol is available for cotton event LLCotton25, based upon nucleotide sequences that are specific to the event.

- Southern blot analysis: Southern blots are used to identify and quantitate specific DNA sequences without amplifying the DNA.

- Protein based-methods specific to PAT protein, such as **ELISA** and **Lateral Flow Strips** are commercially available.

Reference material (specific PCR primers, genomic DNA, seeds) of cotton event LLCotton25 can be provided upon request, and upon agreement with Bayer CropScience.

INFORMATION ON THE POTENTIAL ENVIRONMENTAL IMPACT FROM THE RELEASE OF THE GMHP

29. Potential environmental impact from the release or the placing on the market of GMOs (Annex II, D2 of Directive 2001/18/EC), if different from a similar release or placing on the market of the recipient or parental organism(s)

The following conclusions were drawn (see items listed in Annex IID2 of Directive 2001/18 EC):

1) The herbicide-tolerant cotton neither becomes more persistent than the recipient plant in agricultural habitats, nor shows any changed behaviour with respect to invasiveness in natural habitats.
2) A selective advantage to the herbicide-tolerant cotton could only be identified upon treatment with glufosinate ammonium.
3) Potential for gene transfer to wild and/or cultivated cotton is the same as with non-genetically modified cotton: in Europe the only target for gene flow will be *Gossypium hirsutum* (other cotton crop). The same selective advantage, tolerance to glufosinate-ammonium, would be conferred. Nevertheless LLCotton25 is not intended to be grown in Europe and thus, this risk is hypothetical only.
4) There are no target organisms.
5) No impact could be identified on non-target organisms.
6) No adverse effects on human health from contact or handling have been identified.
7) No adverse effect on animal health or the feed/food chain following animal use has been identified.
8) No effect or alteration on biogeochemical processes was observed.
9) No change in the handling of the imported commodity.

The overall conclusion is that:

- The potential adverse effect identified is pollen dispersal to commercial cotton
- Adequate risk management can be achieved by an isolation of distance of 30 meters – sufficient to prevent outcrossing in commercial cotton.

The overall risk of herbicide-tolerant cotton, taking into account the risk management strategies available, is therefore nil.

30. Potential environmental impact of the interaction between the GMHP and target organisms (if applicable), if different from that of the recipient or parental organism(s)

Not applicable.

31. Possible environmental impact resulting from potential interactions with non-target organisms, if different from that of the recipient or parental organism(s)

a) Effects on biodiversity in the area of cultivation

Under pressure of selection in an area treated with glufosinate-ammonium, LLcotton25 may establish in the environment and, thereby, modify the biodiversity, which could be avoided with good agricultural practices.

Considering that the purpose of this notification is import only, the probability for the occurrence of any type of interaction of LLcotton25 with non-target organisms is very low and in consequence is identical to that experienced from the beginning of the use of cotton commodities in the region.

b) Effects on biodiversity in other habitats

The only habitats where LLcotton25 commodities could occur are harbours, roadsides, processing plants or feed yards. Establishments of volunteers in these habitats are either very unlikely or can be easily controlled by current practices.

c) Effects on pollinators

Not applicable.

d) Effects on endangered species

Not applicable.

C. INFORMATION RELATING TO PREVIOUS RELEASES

32. History of previous releases 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

No part B release in the EU.

b) Conclusions of post-release monitoring

No part B release in the EU.

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*)

No part B release in the EU.

33. History of previous releases carried out inside or outside the Community by the same notifier

a) Inside the Community:

No part B release in the EU.

b) Outside the Community:

Field trials were performed in the USA (USDA authorisations: 99-007-08n, 00-074-14n, 00-108-10n, 00-119-05N, 00-258-02n, 01-075-17n, 01-102-21n, 01-108-05n, 02-039-06n, 01-271-05n, 02-070-21n, 02-107-07n and 02-128-01n), Guatemala, Brazil, South Africa and Australia.

c) Release site:

Field tests have occurred at more than 40 sites in all the regions of adaptation in the USA in the following States: Mississippi, Arizona, Arkansas, California, Texas, Alabama, North Carolina, South Carolina, Florida, Tennessee, Missouri as well as in winter nurseries in Puerto Rico, Guatemala and South Africa, and in Australia and Braazil.

d) Aim of the release:

Trials were initiated with the purpose to complete efficacy-, breeding-, risk assessment-, nutritional composition studies, residue analysis, seed increase and seed production for animal feeding studies.

e) Duration of the release:

In general the cotton was planted depending on the location from February to June and harvest was complete between September to November.

f) Aim of post-releases monitoring:

Data that may provide indications of weediness, and occurrence of volunteers were collected.

g) Duration of post-releases monitoring:

There are no regulatory requirements for post-release monitoring of glufosinate tolerant cotton in the US.

Bayer CropScience continued post-releases monitoring in the subsequent season.

h) Conclusions of post-release monitoring:

The glufosinate tolerance trait did not change the volunteer potential, mode of reproduction, survivability, dissemination risk and generation time of cotton.

i) Results of the release in respect to any risk to human health and the environment:

No adverse affect of LLcotton25 was observed in comparison to conventional cotton varieties.

D. INFORMATION RELATING TO THE MONITORING PLAN - IDENTIFIED TRAITS, CHARACTERISTICS AND UNCERTAINTIES RELATED TO THE GMO OR ITS INTERACTION WITH THE ENVIRONMENT THAT SHOULD BE ADDRESSED IN THE POST COMMERCIALISATION MONITORING PLAN

The environmental risk assessment identifies no risk for the import of glufosinate tolerant cotton commodities that would necessitate carrying out a Case Specific Monitoring.

LLcotton25 will be imported for direct use as food and feed or for processing. No seeds will be imported for cultivation into Europe. The environmental risk for Europe is no greater than the risks associated with the import and processing of cotton in commerce today.

A plan according to Annex VII for the import of genetically modified cotton LLcotton25 has been prepared. This plan will provide for the general surveillance of unanticipated occurrence of adverse effects of the GMHP or its use on human health or the environment, which were not anticipated in the environmental risk assessment.