Approval of Aventis CropScience USA LP Petition (02-042-01p) Seeking a Determination of Non-regulated Status for Glufosinate-ammonium Herbicide-tolerant Cotton Transformation Event LLCotton25

Environmental Assessment and Finding of No Significant Impact

March 2003

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA) has prepared an environmental assessment (EA) prior to approving a petition (APHIS number 02-042-01p) for a determination of nonregulated status received from Aventis CropScience USA LP under APHIS regulations at 7 CFR Part 340. The subject of this petition, LibertyLink® cotton transformation event LLCotton25, is genetically engineered for tolerance to the herbicide glufosinate-ammonium by the insertion and expression of a modified foreign gene encoding the enzyme phosphinothricin acetyltransferase. On December 16, 2002, APHIS published a notice in the Federal Register (67 FR 77034-77035, Docket no. 02-092-1) announcing the availability of the petition and EA for public review and comment. During the designated 60 day comment period, APHIS received two comments, both of which were in favor of a determination of nonregulated status for LLCotton25. APHIS has reached a finding of no significant impact (FONSI) to the environment from its determination that LLCotton 25, and progeny derived from it, shall no longer be considered regulated articles. The determination is included as Appendix E of the EA.

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Date: MAR 1 0 2003

USDA-APHIS Decision on Aventis CropScience USA LP Petition 02-042-01p Seeking a Determination of Nonregulated Status for Glufosinate-Tolerant Cotton Transformation Event LLCotton25

Environmental Assessment

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APPENDICES

I. SUMMARY

The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) has prepared an Environmental Assessment (EA) prior to making its determination on the regulated status of cotton (*Gossypium hirsutum* L.) LibertyLink® transformation event LLCotton25. LLCotton25 has been genetically engineered for tolerance to the herbicide, glufosinate ammonium through the expression of a foreign gene derived from *Streptomyces hygroscopicus*. APHIS received a petition (APHIS number 02-042-01P) from Aventis CropScience USA LP[1] (hereafter referred to as Aventis) for a determination that LLCotton25 does not present a plant pest risk, and therefore should no longer be considered a regulated article under APHIS regulations found at 7 CFR Part 340. The petition submitted by Aventis contains extensive information relevant to this determination. LLCotton25 has been considered a regulated article under APHIS regulations at 7 CFR Part 340 because some DNA regulatory sequences used to control the expression of the foreign gene were derived from plant pests and a plant pest was used as a vector for introduction of the foreign gene.

As a regulated article under the provisions of 7 CFR Part 340, the importation, interstate movement, or field tests of LLCotton25 have been conducted under authorizations from APHIS. These authorizations stipulate conditions of physical and reproductive confinement that preclude the regulated article from becoming mixed with nonregulated articles or persisting in the environment outside the test site.

This EA summarizes the APHIS review of potential environmental impacts that might occur from an APHIS determination that LLCotton25 should no longer be considered a regulated article under the regulations found at 7 CFR Part 340. APHIS received two comments on the petition and the EA during the 60-day comment period announced in the Federal Register (67 FR 77034-77035, Docket no. 02-092-1). The comments were received from a cotton farmer and an organization representing the domestic cotton industry. Both favored a determination of nonregulated status for LLCotton 25. They cited numerous perceived benefits to cotton growers from herbicide tolerant cotton in general, and specific benefits that LLCotton 25 could offer, in conjunction with use of the companion herbicide marketed under the tradename Liberty® (whose approval for use on this cotton is still pending with the US Environmental Protection Agency).

II BACKGROUND

A. Development of LLCotton25

Aventis developed LLCotton25 as a cotton variety with resistance to the nonselective, non-systemic herbicide glufosinate ammonium (GA), which is marketed under the trade name

[1]Since the petition was submitted, Bayer acquired Aventis CropScience, and the new company is now called Bayer CropScience.

Liberty®. LLCotton25 was developed by transforming cotton with the *bar* (bialophos resistance) gene from the bacterium, *Streptomyces hygroscopicus* that encodes the PAT enzyme (Thompson et al., 1987).. This enzyme catalyzes the conversion of L-phosphinothricin, the active ingredient of GA, to an inactive form thereby conferring resistance to the herbicide (OECD, 1999). The gene was introduced by *Agrobacterium*-mediated gene transfer of a fragment of plasmid DNA. Field tests of LLCotton25 were conducted in more than 40 sites from 1999 to 2001 in the United States and Puerto Rico under authorizations granted by APHIS in accordance with the regulations at 7 CFR Part 340 (see Appendix B and petition pg. 46.) These tests were conducted, in part, to confirm that LLCotton25 plants exhibit the desired agronomic and quality characteristics and do not pose a greater plant pest risk than the unmodified cotton variety, Coker 312, from which they were derived. APHIS authorizations stipulate that the regulated article not be planted with nonregulated plant material that is not part of the field release, that it be contained or devitalized when no longer in use, and that the regulated article and its offspring must not persist in the environment after completion of the test. Measures were employed to achieve physical and reproductive confinement from other sexually compatible plants and to manage volunteers.

B. APHIS Regulatory Authority

APHIS regulations under 7 CFR Part 340, which are promulgated pursuant to authority granted by the Plant Protection Act (Title IV, Pub. L. 106-224, 114 Stat. 438, 7 U.S.C. 7701-7772), regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. A genetically engineered organism is considered a regulated article if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation and is also a plant pest, or if there is reason to believe that it is a plant pest. LLCotton25 has been considered a regulated article because plant pathogens served both as a donor for some noncoding DNA regulatory sequences and as a vector to introduce the foreign gene.

Section 340.6 of the regulations, entitled "Petition for Determination of Nonregulated Status", provides that a person may petition the Agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism from which it is derived, the Agency can grant the petition in whole or in part. Therefore, APHIS permits or notifications would no longer be required for field testing, importation, or interstate movement of that article or its progeny.

C. U.S. Environmental Protection Agency (EPA) and Food and Drug Administration (FDA) Regulatory Authority.

The EPA is responsible for the regulation of pesticides, including herbicides, under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended (7 U.S.C. 136 *et seq.*). FIFRA requires that all pesticides, including herbicides, be registered for use on specific crops prior to distribution or sale. Residue tolerances for pesticides are established by the EPA under the Federal Food, Drug and Cosmetic Act (FFDCA), as amended (21 U.S.C. 301 *et seq*.). A pesticide petition (number OF6140) has been submitted to the EPA to expand the current registration of Liberty® herbicide (EPA registration number 264-660) to include use on LibertyLink® cotton. This petition is still pending. The Food and Drug Administration (FDA) enforces tolerances set by the EPA under the FFDCA.

FDA's policy statement concerning regulation of products derived from new plant varieties, including those genetically engineered, was published in the Federal Register on May 29, 1992, and appears at 57 FR 22984-23005. Aventis submitted a summary of their safety assessment to the FDA in August 2002, but they have not yet completed their consultation with the agency.

III. PURPOSE AND NEED

In compliance with the National Environmental Policy Act (NEPA) of 1969 (42 U.S.C. 4321 *et seq.)* and the pursuant implementing regulations (40 CFR 1500-1508, 7 CFR Part lb; 7 CFR Part 372), APHIS has prepared this EA before making a determination on the status of LLCotton25 as a regulated article under APHIS regulations found at 7 CFR Part 340.

IV. ALTERNATIVES

A. No Action: Continuation as a Regulated Article

Under the "no action" alternative, APHIS would not come to a determination that LLCotton25 should no longer be considered a regulated article under 7 CFR Part 340. As such, APHIS authorizations would still be required for introductions, thereby effectively precluding the possible use of this cotton and its progeny in typical commercial farming production. APHIS can choose this alternative if there is insufficient evidence to demonstrate lack of plant pest risk from the unconfined cultivation of LLCotton25 cotton and its progeny.

B. Proposed Action: Determination of Nonregulated Status, in Whole

Under this alternative, APHIS would reach a determination that LLCotton25 and its progeny do not pose a plant pest risk and therefore, should no longer be considered regulated articles under 7 CFR Part 340. With such a determination of nonregulated status, APHIS authorizations would not be required for introductions of this cotton in the United States or its territories. A determination of nonregulated status under 7 CFR Part 340 does not preclude any other requirements or restrictions which might be placed on the use of Liberty® herbicide on these plants by other regulatory agencies (e.g., registration with EPA).

C. Proposed Action: Determination of Nonregulated Status, in Part

The regulations at 7 CFR Part 340.6 (d) (3) (I) state that APHIS may "approve the petition in whole or in part." There are two ways in which a petition might be approved in part:

1. Approval of only some lines requested in a petition. In some petitions, applicants request de-regulation of lines derived from more that one independent transformation event. In these cases, supporting data must be supplied for each line. APHIS could approve certain lines requested in the petition, but not others.

2. Approval of the petition with geographic restrictions. APHIS could determine that the regulated article poses no significant risk in certain geographic areas, but may pose a significant risk in others. In such a case, APHIS might choose to approve the petition with a geographic limitation stipulating that the approved lines could only be grown without APHIS authorization in certain geographic areas.

V. POTENTIAL ENVIRONMENTAL IMPACTS

APHIS considered potential environmental impacts of each of the three alternatives described in Section IV above.

A. Alternative A: No Action

If APHIS takes no action (i.e., does not grant nonregulated status), commercial scale production of LLCotton25 and its progeny is effectively precluded. These plants could still be grown, although still under the requirements of APHIS authorizations (permits or notifications). The plants could be evaluated in field trails for variety development as they have been grown for the past several years. APHIS is unaware of any significant environmental impacts associated with field testing of these plants, and the Agency expects that future field tests under APHIS authorizations would be similar.

With respect to commercial production, APHIS believes that without the option of cultivating LLCotton25 or its progeny, cotton producers would still have the same options available to them for the control of weeds in cotton, including herbicides. It appears likely that the potential environmental impact from continued regulated status of LLCotton25 would not be significant. Cotton farmers would continue to use existing technologies for the control of weeds.

The development of varieties based on LLCotton25 and its progeny could increase weed control options to growers if the EPA also grants the requested pesticide petition to allow use of Liberty® on LibertyLink® cotton. However, granting nonregulated status does not guarantee the extent to which a new plant line, such as LLCotton25, would be adopted by growers. As a regulated article, the field testing of LLCotton25 plants could continue under APHIS authorizations (permits or notifications), but commercial scale production would not be feasible. APHIS does not foresee significant impacts to the environment if Alternative A is chosen.

B. Alternative B: Approval of the Petition in Whole

APHIS may grant a petition for nonregulated status in whole or in part. By granting the petition in whole, APHIS grants the petition as requested for LLCotton25 without geographical restrictions. The APHIS assessment of environmental impacts of such a determination are discussed in the following sections. Environmental impacts of unrestricted cultivation of LLCotton25 are compared to impacts of current practices in the cultivation or distribution of cotton not regulated under 7CFR part 340.

1. Plant pathogenic properties

APHIS considered the potential for the transformation process, the introduced DNA sequences or their expression products to cause or aggravate disease symptoms in LLCotton25 or in other plants, or to cause the production of plant pathogens. We also considered whether data indicate that unanticipated plant pest effects would arise from cultivation of LLCotton25. APHIS considered information from the scientific literature as well as primary observations made by the developer when the plants were grown in the environment.

Recipient organism
The starting plant material for the transformation was derived from cotton variety Coker 312 (PVP 7200100) of SEEDCO Corporation, Texas. Coker 312 was developed from a cross of Coker 100 x D&PL-15 and selected through successive generations of line selection.

Transformation system
The transformation system for LLCotton25 employed *Agrobacterium*-mediated transformation technology that utilized pGSV71, a binary plasmid vector carrying the *bar* gene construct within a disarmed transfer DNA (T-DNA) from *Agrobacterium tumefaciens* that lacks the hormone genes from this pathogen that cause crown gall disease. *Agrobacterium*-mediated transformation is a well characterized technique that has been used for the transformation of plant cells for over a decade.

DNA sequences introduced to make LLCotton25
The Aventis petition provided data to support the conclusion that LLCotton25 contains the T-DNA insert (petition, pages 25-33, and May 7, 2002 Addendum). The inserted DNA consists of the following sequences: A portion of the right border repeat from the TL-DNA from plasmid pTIB6S3, a synthetic polylinker sequence, the 35*S* promoter of cauliflower mosaic virus (CaMV), the coding sequence for the *bar* gene[2] from *S. hygroscopicus*, a synthetic polylinker sequence, a fragment from the 3' untranslated end of the nopaline synthase gene (3' *nos*) from the T-DNA of plasmid pTiT37 from *A. tumefaciens*, a synthetic polylinker sequence, and the left

[2]The *bar* coding sequence was modified to include a two amino acid substitution at the amino-terminal end (see petition pg. 20).

border repeat from the TL-DNA from PTiB6S3. Aventis presented data to confirm that sequences of the plasmid backbone were not introduced when LLCotton25 was made (see petition text page 29).

Of all of the DNA sequences inserted in the construction of LLCotton25, only the *35S* promoter and the 3' *nos* termination sequence were derived from organisms known to be plant pests (CaMV and *A. tumefaciens*, respectively). These noncoding sequences are well characterized, both in their native organisms and as part of recombinant DNA constructs used in plant engineering so that introduced genes can be expressed and their transcripts (mRNA) correctly processed. There are no data to suggest that the *35S* promoter or the *nos* terminator sequences cause plant disease or pose a plant pest risk in transgenic plants. Multiple generations of LLCotton25 plants have been observed closely, and the developer has confirmed the expectation that these noncoding DNA sequences do not cause disease in the plants (see sections below for discussion of additional evaluations of the attributes of LLCotton25 plants).

None of the other donor organisms used as sources for the DNA sequences engineered into the cotton to make LLCotton25 are organisms with demonstrated plant pest characteristics. *S. hygroscopicus* (strain ATCC21705) is a gram-positive, sporulating soil bacterium.

Evaluation of intended effects in LLCotton25:
As intended, LLCotton25 expresses the phosphinothricin acetyltransferase (PAT) protein encoded by the *bar* gene. The Aventis petition summarized data which demonstrates the expression of this protein in plant tissues sufficient to confer the desired GA resistance trait. Expression of the PAT protein was also detectable in all fractions of transgenic fuzzy seed and lint. PAT protein content varied between different trial sites and between treatments with Liberty® (Page 44 of the petition).
The Aventis petition also summarized data which demonstates that the GA resistance trait conferred by this protein is inherited in a predictable manner when LLCotton25 plants are crossed with other cotton plants (see petition page 25 for Mendelian inheritance data; and the August 9, 2002 Addendum). The petition provided data on field tests of LLCotton25 in which the plants exhibited resistance to Liberty® herbicide at application rates of 1x (28 oz/acre) and 4x. These field tests took place in 40 field trial locations conducted from 1999 through 2001 to evaluate various parameters in addition to resistance to Liberty®, including emergence, seedling vigor, stand establishment and maturity (pages 45 and 46 of the petition).

Evaluation of possible unintended effects in LLCotton25
Expression of the PAT protein is not expected to cause plant disease or influence the susceptibility of LLCotton25 to plant pathogens or pests. Aventis evaluated the expression levels of this protein in LLCotton25 plants growing in the field and confirmed that the plants were no more susceptible to pathogens and pests of cotton. In field tests, no differences were noted for disease susceptibility or severity in the LLCotton25 plants compared to the control cotton plants that had no PAT gene (Petition pp. 49 and 51).

In order to evaluate possible unintended effects of the transformation process, including effects from tissue culture, APHIS considers a wide range of plant attributes in much the same way that traditional plant breeders evaluate the offspring from traditional plant crosses or mutagenesis procedures. The petition included extensive information on the attributes of LLCotton25. Observations were made from seedling emergence through maturity on LLCotton25 plants grown from 1999 through 2001 in more than 40 sites distributed in Mississippi, Arkansas, North Carolina, Texas, Tennessee, Missouri, Alabama, Georgia, Louisiana, South Carolina and in winter nurseries in Puerto Rico (petition pp. 45-46). These states are among the top states in total cotton acreage planted in the United States (NASS, 2000). In 2000 and 2001, fifth and sixth generation (T5 and T6) LLCotton25 plants were compared to the nontransgenic parent cotton variety Coker 312. Evaluations in 2001 also compared LLCotton25 in six different genetic backgrounds to their respective nontransgenic recurrent parent cotton variety counterparts. In both years, nontransgenic control varieties were treated with conventional herbicide regimes typical for cotton cultivation in the United States and the LLCotton25 varieties were treated with conventional herbicide regimes (no Liberty®) or with one and/or two applications of Liberty® herbicide at the 1x or 4x rate.

Field observations were made by Aventis field researchers (breeders, agronomists, entomologists, field cooperators) who are very familiar with cotton agronomic properties and cultivation of the crop. In addition to herbicide tolerance efficacy ratings, plants were evaluated for disease/pest susceptibility, plant growth and morphology parameters (e.g. stand count, seedling vigor, plant height, height to node ratio, sympodia length, leaf morphology, overall plant morphology), various reproductive traits, productivity in yield of seed and lint, and various fiber quality parameters (see pages 49 and 50 in the Petition). In general no differences were noted between LLCotton25 and the nontransgenic parent Coker 312 that could be attributed to the transformation process. Some differences in plant maturity were noted as reflected in the values for days to first bloom at one of the sites in 2000, but this was variable depending on the herbicide regime (Petition pg. 101). As expected, differences were observed in 2001 between the different genetic backgrounds for some parameters of yield, maturity, and fiber quality, but significant differences within a genetic background between LLCotton25 transgenics and nontransgenic controls were only observed for fiber quality and stand counts (Petition pg. 115). One LLCotton25 line had improved fiber uniformity and one line had lower strength. Three LLCotton25 lines had lower stand counts, but this effect was not noted in the 2000 trials. Overall, the field observations support the conclusion that LLCotton25 is typical of traditional cotton in terms of growth and agronomic performance.

Aventis also analyzed for potential changes in the proximate composition (protein , fat, moisture, fiber, carbohydrates, and ash) of the seeds and lint, and the key mineral, vitamin E, and antinutrient composition of the cotton seed and found no significant differences in analyses between LLCotton25 treated with Liberty® herbicide or conventional herbicides and that of the parental cultivar, Coker 312 treated with conventional herbicides (Petition, pages 53-56). The antinutrient analysis included gossypol, phytic acid, and cyclopropanoid fatty acids. Gossypol is a known toxicant normally produced by cotton plants that is believed to protect them from being eaten by insects and herbivorous mammals.

The only consistent significant difference in plant attributes observed across years and locations between LLCotton25 (both in the Coker 312 genetic background and other genetic backgrounds) and the nontransformed counterparts, was the intended ability of LLCotton25 to resist application of GA herbicide. These observations provide further evidence that LLCotton25 has not been modified in unintended ways in the course of transformation, plant generation, and traditional plant breeding. APHIS can not envision any plant pest effects arising from a determination that LLCotton25 should no longer be considered a regulated article under the APHIS regulations found at 7CFR Part 340.

2. Potential Impacts based on the relative weediness of LLCotton25 compared to currently cultivated cotton varieties.

APHIS evaluated whether LLCotton25 would be any more likely to become a weed than the parental line Coker 312, or than other cotton varieties currently offered for commercial use. The cultivated cotton from which line Coker 312 is derived, *Gossypium hirsutum*, is not typically considered a weed species in the United States or other countries (Reed, 1977; Muenscher, 1980; Holm et al., 1977, 1997; USDA, NRCS. 2001) nor is it listed in the Weed Science Society's Composite List of Weeds (1989). However, the Southern Weed Science Society lists *G. hirsutum* as a potential weed in southern Florida (Southern Weed Science Society, 1998). Without human intervention, such as the typical agricultural practices, the cotton plant is a perennial, surviving many years if conditions allow. Cotton does not tolerate cold conditions, and only Hawaii, southern Florida, and Puerto Rico remain warm enough to allow cotton plants to survive the winter. Cotton has some characteristics as a weed, and it has been identified as one in southern Florida.

As described above, APHIS evaluated quantitative and qualitative data submitted in the Aventis petition that substantiated that LLCotton25 derived lines were similar to nontransgenic counterpart varieties when grown over a variety of locations and years, with or without Liberty® herbicide, for a number of parameters, some of which might be predictive of weediness, fitness, competitiveness, fecundity, or survival (Baker, 1974). These include plant growth and morphology characteristics described previously, reproductive characteristics (e.g. days to first bloom and to 50% open bolls, fertility, seed index, and number of seeds per boll), lint and seed yield parameters, disease and pest susceptibility, and seed antinutrient composition. Although stand counts (seed germination measured after 28 days in the field) were lower for LLCotton25 in some locations, seedling vigor was not significantly different. Seed germination data provided in Appendix C of the petition indicated that no difference in germination rates under normal regimes were seen in LLCotton25 and Coker 312 seeds collected from five locations. But an overnight cold treatment, which is sometimes used to increase germination rates in cotton seed, actually reduced germination of LLCotton25 compared to the nontransgenic Coker 312 parent. However, the petitioner noted in the May 7 Addendum that this was not observed in previous years. Even if seed germination of LLCotton25 was sensitive to prior cold treatment, this would make it even less competitive in cold environments.

In addition to the results summarized above, APHIS notes that there have been no reports of increased weediness associated with the plant that is most similar to LLCotton25, namely its parent Coker 312. On the basis of all the submitted data and field observations to date, LLCotton25 appears to pose no greater plant pest risk of weediness than that posed by traditional cotton cultivars.

3. Potential impacts from gene introgression from LLCotton25 to its sexually compatible relatives.

LLCotton25, like other cotton, can pass its traits to offspring by transmitting pollen to other plants which are sexually compatible, in this case, to some species of the genus *Gossypium* (see Appendix A of this environmental assessment for a brief technical discussion of the biology and reproductive capability of cotton). Recently, EPA has provided an even more detailed overview of the genus *Gossypium* in the Biopesticides Registration Action Document (http://www.epa.gov/pesticides/biopesticides/reds/brad_bt_pip2.htm, see especially pages IIC7-IIC13 in US EPA, 2001).

APHIS considered whether such crosses are likely to occur when LLCotton25 is grown, and whether the offspring from such crosses are more likely to pose any greater risk of weediness than crosses of other cotton cultivars with these sexually compatible species.

The genus *Gossypium* contains 39 species, of which generally four species are cultivated for the cotton fibers that are attached to the seeds. LLCotton25 is *Gossypium hirsutum*, the cotton species referred to as upland cotton. Most of the cotton grown in the United States is *G. hirsutum*, but Pima cotton (*G. barbadense* L.) is also grown. In addition to these cultivated species, there are two wild *Gossypium* species in the United States, *G. thurberi* and *G. tomentosum*, which are found in parts of Arizona and Hawaii, respectively. Neither *G. thurberi* nor *G. tomentosum* are listed as weeds, either on the Federal or State lists of noxious weeds (see http://plants.usda.gov/cgi_bin/noxious.cgi?earl=noxious.cgi). An older literature citation lists *G. tomentosum* as a weed of unknown importance in its range (Holm et al.,1979).

Genetic incompatibility precludes successful crosses of *G. hirsutum* with *G. thurberi*, but the compatibility of crosses between *G. hirsutum* and *G. tomentosum* is more unknown. Some researchers have speculated that crosses may have occurred in the evolution of *G. tomentosum*, but genetic exchange appears to be rare. Part of the rarity may be due to the fact that *G. hirsutum* is largely self-pollinating rather than cross-pollinating. In addition, the pollinators of *G. hirsutum* tend to be bumblebees, whereas moths pollinate *G. tomentosum*. Also, *G. hirsutum* flowers are sexually receptive for pollination during the day, whereas *G. tomentosum* compatibility is at night.

Even in cases of complete genetic compatibility (*G. hirsutum* crossed with another *G. hirsutum*), successful outcrossing is severely limited when the plants are separated by more than 660 feet. In experiments designed to detect gene flow, detectable gene flow was very low (less than 1%) when *G. hirsutum* plants were 25 meters apart (Umbeck, 1991). Cotton breeders and seed producers routinely use field data to decide on the isolation distances for the production of certified and foundation cotton seeds (660 and 1320 feet, respectively). APHIS evaluated data submitted in the Aventis petition that substantiates that no consistent significant differences were observed between LLCotton25 and the nontransgenic parent Coker 312 in reproductive traits measured under numerous field conditions. Nor were there significant differences in flower morphology, or viability and germination of pollen in greenhouse-grown plants (Petition, Appendix F, pp 174 - 192). Therefore, there is no reason to suspect that LLCotton25 would have a greater outcrossing rate.

In sum, APHIS believes that it is very unlikely that LLCotton25 will successfully cross with wild sexually compatible relatives when grown in the United States. In the unlikely event that such crosses do occur, however, the lack of increased weediness of LLCotton25 (described in the section above) suggests that any offspring would be unlikely to pose an increased risk of weediness.

Because it is unlikely that *G. hirsutum* will readily cross with *G. thurberi* and *G. tomentosum*, it is unlikely that the *bar* gene will introgress from LLCotton25 into *G. thurberi* and *G. tomentosum*. In the registration requirements for the first generation of BT-cotton varieties, the EPA stipulated geographic restrictions in parts of the United States where *G. thurberi* and *G. tomentosum* are found, imposing conditions based on reproductive compatibility in crosses of *G. hirsutum* to other *G. hirsutum*. As summarized above, however, such crosses between the cultivated and wild cottons do not appear to occur in nature. There are no reports of intermediate cotton types that one would expect in the areas where *G. hirsutum* has been grown in proximity to *G. thurberi* and *G. tomentosum*.

Outcrossing considerations may be different in other parts of the world. For example, other species which might potentially intercross with *G. hirsutum* cultivars include *G. mustelinum* in northeastern Brazil, and *G. lanceolatum* in mid-Mexico (Fryxell 1979). Other Old World Gossypium cottons are diploid, as are the other five genera of cotton relatives among the Gossypieae Tribe (Fryxell, 1979). The likelihood of successful intercrossing with these species may be quite low because of the production of triploids that are likely to be sterile. This is consistent with the fact that such intergeneric crosses have not been observed (Fryxell, 1979).

APHIS believes that gene flow from LLCotton25 to wild cotton relatives is not likely, and if it occurs, would not lead to to increased weediness. APHIS agrees with the EPA statement in its final rule on plant-incorporated protectants (66 FR 37772-37817, July 19, 2001) that "weediness is generally thought to be due to a multiplicity of factors". The National Research Council came to the same conclusion that "genetically modified crops are not known to have become weedy through the addition of traits such as herbicide and pest resistance" (National Research Council, 1989).

4. Potential impacts on nontarget organisms, including beneficial organisms and threatened and endangered species

APHIS evaluated the potential that LLCotton25 might have an impact on populations of nontarget organisms or species which are recognized or proposed as threatened or endangered by the U.S. Fish and Wildlife Service. Field data reports submitted by Aventis for the numerous field trials conducted between 1999 and 2001, indicated that no toxicity or alteration of population levels were observed for beneficial insects, birds or other species that frequent cotton fields. There were no qualitative differences in beneficial species and populations present on transgenic and non-transgenic cotton plants. This is supported by data cited in the petition that indicate that the PAT enzyme is highly specific to the target herbicide, it occurs in low levels in the plant tissue, is rapidly degraded in vitro in gastric fluid similar to livestock, and it is neither toxic nor allergenic (Petition pg. 59). Furthermore, as mentioned above, the toxicant and antinutrient composition of LLCotton25 cotton seed was not significantly different from that of the nontransformed parent Coker 312, nor was it outside the normal range reported in the literature. As documented in environmental assessments for previous petitions submitted for other GA (phosphinothricin) tolerant crops, APHIS has never encountered impacts on nontarget organisms associated with the expression of PAT. Cotton (*G. hirsutum*) is not sexually compatible with any plant species listed as threatened or endangered. The genetic modification in LLCotton25 is not expected to increase its ability to grow in new habitats, so it would not be expected to displace any threatened or endangered plant species. For these reasons, no effect on nontarget organisms, including those on the Federal List of Threatened and Endangered Species, is expected.

The adoption of cotton varieties derived from glufosinate-tolerant cotton LLCotton25 into cotton production may result in a shift in the application of herbicides currently used for weed control in cotton, if the EPA also grants the requested pesticide petition to allow the use of Liberty® herbicide on LibertyLink® cotton. This shift may result in differences in impacts to nontarget species of plants or animals via spray drift, bioaccumulation in food chains, and the contamination of surface and groundwater sources depending on the toxicity profile of the herbicides and their metabolites. The EPA will address this issue when they evaluate the impacts of a decision on this pesticide petition.

5. Potential Impacts on Biodiversity

After careful evaluation, APHIS believes that LLCotton25 exhibits no traits that would cause increased weediness, its cultivation should not lead to increased weediness of other cultivated cotton or other sexually compatible relatives, and it is unlikely to harm non-target organisms common to the agricultural ecosystem or threatened or endangered species recognized by the U.S. Fish and Wildlife Service. Based on this analysis, APHIS believes that it appears unlikely that LLCotton25 will pose a significant impact on biodiversity.

6. Potential Impacts on Agricultural and Cultivation Practices

APHIS considered the potential impacts of LLCotton25 on current agricultural practices in the United States, including organic farming. APHIS also considered any potential cumulative effects that might arise from the use of LLCotton25 or its progeny in agricultural production. Potential impact on minorities, low income populations, and children were also considered.

Impacts on current agricultural practices

APHIS considered information provided in the petition (Petition pp. 59-64) regarding past and current weed control practices in cotton and the intended and potential impacts that could result from a determination of nonregulated status for LLCotton25 and the potential expanded registration of the Liberty® herbicide for use on LibertyLink® cotton.

A variety of herbicides and cultivation practices are recommended for weed control in cotton (Vargas et al., 2001). Recently cotton varieties tolerant to other herbicides (those resistant to the broadleaf herbicide bromoxynil or to the broadspectrum herbicide glyphosate) have been grown over fairly large acres in the United States. This shift to herbicide tolerant varieties is associated with a significant reduction in the number of herbicide applications, a reduction in the total amount of active ingredient of herbicides applied, and a shift from soil-applied and more persistent herbicides to those that are applied post-emergent, over the top. In 1999 and 2000, bromoxynil tolerant cotton was planted on about 8% of U.S. cotton acres, and in 2000 - 2001, glyphosate (Roundup®) tolerant cotton made up greater than 50% of U.S. cotton acres. The introduction of a selective broad-leaf herbicide, pyrithiobac (Staple) in 1995, that can be applied at any stage of cotton growth and has low application rates, may also have contributed to the recent decline in active ingredients applied (Bruening, 2002, and references therein). Currently, more than 97% of cotton acres receive some herbicide treatment, typically this may consist of a pre-plant herbicide, followed by a post-emergent herbicide. Of the post-emergent herbicides, glyphosate currently makes up 37% of the market and and pyrithiobac, MSMA and bromoxynil make up 11% of the market (petition, pg. 60).

Glufosinate ammonium is currently registered under various trade names for control of weeds in other crops, and as Liberty® for use on LibertyLink® corn and canola varieties. It acts by inhibiting the enzyme glutamine synthase which causes a toxic buildup of ammonia within the weed (OECD, 1999). This herbicide has a different mode of action than the other major herbicides used in cotton that it is intended to replace or supplement, and unlike the other herbicides, there are no weed biotypes with resistance reported to this class of herbicide in an international survey of herbicide resistant weeds (Heap, 2002). There are at least eight weed species in cotton-producing areas or states that have developed resistance to one or more of the alternative classes of herbicides (e.g. the preplant herbicides trifluralin, pendamethalin, and fluometuron; and post-emergent herbicides pyrithiobac and MSMA) that are also included on the list of important weeds labeled for control by Liberty® in cotton (see Appendix C of this EA). If the Liberty® registration is expanded to include use on LibertyLink® cotton, then the possible commercial use of varieties based upon LLCotton25 may have positive impacts on current agricultural practices. It could provide an opportunity to use Liberty® as an alternative broad-spectrum, post-emergent herbicide in cotton with a wide application window that can allow for more accurate assessment of weed pressure and treatment as necessary. This may reduce the need for some preplant herbicide applications, and provide control of some herbicide resistant weed populations. Corn, cotton, or soybean varieties resistant to glyphosate are currently being widely adopted in the United States (Economic Research Service, 2002). If varieties based upon LLCotton25 are grown in rotation with glyphosate-resistant crop varieties, then Liberty® could be used to control glyphosate-resistant volunteer crops, and it could potentially be used to manage shifts in weed species that can arise from continuous use of a given herbicide. At the same time, volunteers of LLCotton25 based varieties can potentially be controlled by a number of herbicides used in crops grown in rotation with cotton (August 9, 2002 Addendum to the petition). Cultivation of cotton resistant to different herbicides in adjacent fields could lead to the development of cotton volunteers with multiple herbicide resistance, but given the relatively low out-crossing rates in cotton and use of alternate herbicides and/or tillage practices, these should not be a persistent or serious management problem. APHIS notes that the US EPA, Office of Pesticide Programs has issued voluntary pesticide resistant management labeling guidelines based on mode/target site of action for agricultural uses of pesticides, including herbicides in their Pesticide Registration (PR) Notice 2001-5 available on the internet at http://www.epa.gov/opppmsd1/PR_Notices/pr2001-5.pdf. This document also provides information and resources that could be useful for growers seeking to reduce or manage the potential for herbicide-resistant weeds or volunteers.

Potential impacts on organic farming

The National Organic Program (NOP) administered by USDA's Agricultural Marketing Service (AMS) requires organic production operations to have distinct, defined boundaries and buffer zones to prevent unintended contact with prohibited substances from adjoining land that is not under organic management. Organic production operations must also develop and maintain an organic production system plan approved by their accredited certifying agent. This plan enables the production operation to achieve and document compliance with the National Organic Standards, including the prohibition on the use of excluded methods. Excluded methods include a variety of methods used to genetically modify organisms or influence their growth and development by means that are not possible under natural conditions or processes.

Organic certification involves oversight by an accredited certifying agent of the materials and practices used to produce or handle an organic agricultural product. This oversight includes an annual review of the certified operation's organic system plan and on-site inspections of the certified operation and its records. Although the National Organic Standards prohibit the use of excluded methods, they do not require testing of inputs or products for the presence of excluded methods.

The presence of a detectable residue of a product of excluded methods alone does not necessarily constitute a violation of the National Organic Standards. The unintentional presence of the products of excluded methods will not affect the status of an organic product or operation when the operation has not used excluded methods and has taken reasonable steps to avoid contact with the products of excluded methods as detailed in their approved organic system plan. Organic certification of a production or handling operation is a process claim, not a product claim.

It is not likely that organic farmers, or other farmers who choose not to plant transgenic varieties or sell transgenic grain, will be significantly impacted by the expected commercial use of this product since: (a) nontransgenic cotton will likely still be sold and will be readily available to those who wish to plant it; (b) farmers purchasing seed will know this product is transgenic because it will be marketed and labeled as glufosinate resistant.

Several transgenic cotton varieties that are either insect or herbicide resistant are already in widespread use by farmers. Varieties derived from LLCotton25 should not present new and different issues with respect to impacts on organic farmers. APHIS has considered that although cotton is primarily self-pollinated, it is possible that the genes from LLCotton25 could move to cotton in an adjacent field through insect vectored cross-pollination. All cotton, whether genetically engineered or not, can transmit pollen to nearby fields. As described previously in this assessment, the rate of cross-pollination from one field to another is expected to be quite low, even if flowering times coincide. The frequency of such an occurrence decreases with increasing distance from the pollen source such that it sufficiently low at 1320 feet away to be considered adequate for production of even the most restrictive standard for foundation cotton seeds (see footnote 19 for the table found at http://www.aphis.usda.gov/biotech/isolate.html). A very small influx of pollen originating from a given cotton variety does not appreciably change the characteristics of cotton in adjacent fields.

Potential impacts on humans, including minorities, low income populations, and children
Under Executive Order 13045, APHIS has attempted to identify and assess environmental health or safety risks that might disproportionately affect children. APHIS also considered any possible adverse impacts on minorities and low-income populations as specified under Executive Order 12898 published February 11, 1994. Collectively, the available mammalian toxicity data, along with the history of safety of the *bar* gene and its PAT protein, support the safety of LLCotton25 and its products to humans, including minorities, low income populations, and children who might be exposed to them through agricultural production and/or processing. APHIS can not envision what additional safety precautions would need to be taken in consideration of these groups. None of the impacts on agricultural practices described above are expected to have a disproportionate adverse effect on minorities, low-income populations, or children. Should Liberty® herbicide registration be expanded to include use on LibertyLink® cotton, cultivation of glufosinate-tolerant cotton varieties derived from LLCotton25 on a commercial scale could potentially reduce applications of some herbicides with different target specificities and thus may reduce the exposure to them, but it may also result in increased use of GA. The use of herbicides in cotton cultivation are regulated by the EPA. Tolerance levels are established by taking into account the cumulative exposure to the herbicide on all crops for which the herbicide is to be registered. EPA reviews the use of herbicides and it is expected that EPA and the

Economic Research Service of the USDA would monitor the use of this product to determine impacts on agricultural practices.

7. Potential impacts on raw or processed agricultural commodities.

Our analysis of data on agronomic performance, disease and insect susceptibility, and compositional profiles of the seeds and fiber indicate no significant differences between LLCotton25 and its parent and other cultivars of *G. hirsutum* grown in the United States. APHIS does not foresee either a direct or indirect plant pest effect on any raw or processed plant commodity.

8. Potential environmental impacts outside the United States.

APHIS has also considered potential environmental impacts outside the United States and its territories associated with a determination of nonregulated status for LLCotton25. Any international traffic in cotton subsequent to this determination would be fully subject to national phytosanitary requirements and be in accordance with phytosanitary standards developed under the International Plant Protection Convention (IPPC). The IPPC has set a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (116 countries as of June, 2001). In addition, issues that may relate to commercialization of particular agricultural commodities produced through biotechnology are being addressed in international forums. APHIS continues to play a role in working toward harmonization of biosafety and biotechnology guidelines and regulations, including within the North American Plant Protection Organization (NAPPO), which includes Mexico, Canada, and the United States. NAPPO's Biotechnology Panel advises NAPPO on biotechnology issues as they relate to plant protection. APHIS also participates regularly in biotechnology policy discussions at forums sponsored by the European Union and the Organization for Economic Cooperation and Development. APHIS periodically holds bilateral or quadrilateral discussions on biotechnology regulatory issues with other countries, most often Canada and Mexico, and has participated in numerous conferences intended to enhance international cooperation on safety in biotechnology. APHIS has sponsored several workshops on safeguards for planned introductions of transgenic crops most of which have included consideration of international biosafety issues. Mexico and Brazil, both of which have relatives of cotton that can potentially interbreed with it, have procedures in place that require a full evaluation of transgenic plants before they can be introduced into the environment. It should also be noted that all the considerable, existing national and international regulatory authorities and phytosanitary regimes that currently apply to introductions of new cotton cultivars internationally, apply equally to those covered by an APHIS determination of nonregulated status under 7 CFR Part 340.

C. Alternative C, Approval of the Petition in Part

1. Approval of some, but not all, of the lines requested in the petition. The petition requested a determination of nonregulated status only for LLCotton25 and any progeny lines derived from it by traditional breeding practices. Therefore, APHIS can consider only LLCotton25 for approval.

2. Approval of the petition with geographic restrictions. EPA is currently reviewing the petition to include the use of Liberty® on LibertyLink® cotton, such as varieties derived from LLCotton25. EPA has the authority to impose geographic limitations on the use of specific pesticides, including herbicides, and routinely does so to protect threatened and endangered species, as well as other non-target organisms. EPA and APHIS agree that the threatened and endangered species do not typically feed on cotton. APHIS has not identified any potential effects from LLCotton25 on nontarget organisms, including threatened or endangered species or any adverse impacts on related plant species or plant pest effects that would warrant placing geographic restrictions on planting of LLCotton25 by granting the petition in part.

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Appendix A: Biology of cotton and potential for introgression into related species.

Cotton as a Crop

Four species of the genus *Gossypium* are known as cotton, which is grown primarily for the seed hairs that are made into textiles. Cotton is predominant as a textile fiber because the mature dry hairs twist in such a way that fine, strong threads can be spun from them. Other products, such as cottonseed oil, cake, and cotton linters are byproducts of fiber production.

Cotton, a perennial plant cultivated as an annual, is grown in the United States mostly in areas from Virginia southward and westward to California, in an area often referred to as the Cotton Belt (McGregor, 1976).

Taxonomy of Cotton

The genus *Gossypium*, a member of the Malvaceae, consists of 39 species, four of which are generally cultivated (Fryxell, 1984). The most commonly cultivated species, *G. hirsutum* L., is the subject of this Environmental Assessement. Other cultivated species are *G. arboreum* L., *G. barbadense* L., and *G. herbaceum* L.

Four species of *Gossypium* occur in the United States (Fryxell, 1979; Kartesz and Kartesz, 1980). *Gossypium hirsutum* is the primary cultivated cotton. *Gossypium barbadense* is also cultivated. The other two species, *G. thurberi* Todaro and *G. tomentosum* Nuttall ex Seemann, are wild plants of Arizona and Hawaii, respectively. *Gossypium tomentosum* is known from a few strand locations very close to the ocean.

Genetics of Cotton

At least seven genomes, designated A, B, C, D, E, F, and G, are found in the genus (Endrizzi, 1984). Diploid species (2n=26) are found on all continents, and a few are of some agricultural importance. The A genome is restricted in diploids to two species (*G. arboreum*, and *G. herbaceum*) of the Old World. The D genome is restricted in diploids to some species of the New World, such as *G. thurberi*.

By far, the most important agricultural cottons are *G. hirsutum* and *G. barbadense*. These are both allotetraploids of New World origin, and presumably of ancient cross between Old World A genomes and New World D genomes. How and when the original crosses occurred have been subject to much speculation. Euploids of these plants have 52 somatic chromosomes, and are frequently designated as AADD. Four additional New World allotetraploids occur in the genus, including *G. tomentosum*, the native of Hawaii. *Gossypium tomentosum* has been crossed with *G. hirsutum* in breeding programs.

The New World allotetraploids are peculiar in the genus, because the species, at least in their wild forms, grow near the ocean, as invaders in the constantly disturbed habitats of strand and associated environs. It is from these "weedy" or invader species that the cultivated cottons developed (Fryxell, 1979).

Pollination of Cotton

Gossypium hirsutum is generally self-pollinating, but in the presence of suitable insect pollinators can exhibit cross pollination. Bumble bees (*Bombus* spp.), Melissodes bees, and honey bees (*Apis mellifera*) are the primary pollinators (McGregor, 1976). Concentration of suitable pollinators varies from location to location and by season, and is considerably suppressed by insecticide use. If suitable bee pollinators are present, distribution of pollen decreases considerably with increasing distance. McGregor (1976) reported results from an experiment in which a cotton field was surrounded by a large number of honey bee colonies, and movement of pollen was traced by means of fluorescent particles. At 150 to 200 feet, 1.6 percent of the flowers showed the presence of the particles. The isolation distance for Foundation, Registered, and Certified seed in 7 CFR Part 201 is 1320 feet, 1320 feet, and 660 feet, respectively.

Research in Mississippi shows that pollen movement decreases rapidly after 40 feet (12 meters). Umbeck et al. (1991) studied pollen and successful gene movement of cotton in Mississippi test plots. Around a central transgenic test plot of 98,800 plants with rows running north-south, they planted 23 one-meter border rows of nontransgenic cotton to the east and to the west, and 25 meters of non transgenic cotton border rows to the north and to the south, each divided into two 12.5 meter long plots. The border rows to the north and south were continuous with the transgenic rows. They took 32,187 seed samples from all border rows at bottom, middle, and top plant position (representing seasonal variation) and used a kanamycin resistance marker gene to test for seeds resulting from pollen movement out of the central transgenic plot. To the east and west, gene movement at the first row was 0.057 and 0.050, and dropped rapidly to row 8, and was not detected in subsequent rows to the east, and detected occasionally at <0.01 in rows to the west. Combined data for east and west border rows beyond row 9 gave total outcrossing of 0.0012. To the north and south, detections were totaled for each 12.5 meter block and gave figures of 0.0053 and 0.0047 for north and south inner block and 0.0015 and 0.0021 for north and south outer block.

Gossypium tomentosum seems to be pollinated by lepidopterans, presumably moths (Fryxell, 1979). The stigma in *G. tomentosum* is elongated, and the plant seems incapable of self-pollination until acted upon by an insect pollinator. The flowers are unusual too, because they stay open at night; most Gossypium flowers are ephemeral: they open in the morning and wither at the end of the same day.

Weediness of Cotton

Although the New World allotetraploids show some tendencies to "weediness" (Fryxell, 1979), the genus shows no particular weedy aggressive tendencies.

Modes of Gene Escape in Cotton

Genetic material of *G. hirsutum* may escape from a test area by vegetative material, by seed, or by pollen. Propagation by vegetative material is not a common method of reproduction of cotton. Physical safeguards that inhibit the movement of vegetative material from the area should be adequate to prevent gene movement by this means. Movement of seed from the test area can likewise be inhibited by adequate physical safeguards. Movement of genetic material by pollen is possible only to those plants with the proper chromosomal type, in this instance only to those allotetraploids with AADD genomes. In the United States, this would only include *G. hirsutum*, *G. barbadense*, and *G. tomentosum*. *Gossypium thurberi*, the native diploid from Arizona with a DD genome, is not a suitable recipient. Movement to *G. hirsutum* and *G. barbadense* is possible if suitable insect pollinators are present, and if there is a short distance from transgenic plants to recipient plants. Physical barriers, intermediate pollinator-attractive plants, and other temporal or biological impediments would reduce the potential for pollen movement.

Movement of genetic material to *G. tomentosum* is more unknown. The plants are chromosomally compatible with *G. hirsutum*, but there is some doubt as to the possibility for pollination. The flowers of *G. tomentosum* seem to be pollinated by moths, not bees. And they are receptive at night, not in the day. Both these factors would seem to minimize the possibility of cross-pollination. However, Fryxell (1979) reports that *G. tomentosum* may be losing its genetic identity from introgression hybridization of cultivated cottons by unknown means.

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Appendix B. List of confined field tests of LLCotton25 conducted under APHIS authorizations.

USDA Authorization Number	Year Planted	Number of Locations	States or Territories of Locations
99-007-08n	1999	1	MS
00-074-14n	2000	5	AR, NC, MS, TX
00-108-10n	2000	4	TN, MS
00-119-05n	2000	3	MO, MS, TX
00-258-02n	2000	1	Puerto Rico
01-075-17n	2001	26	AL, AR, GA, LA, MS, NC, SC, TN, TX
01-102-21n	2001	1	TX
01-108-05n	2001	1	GA
01-271-05n	2001	1	Puerto Rico

Appendix C. - Resistance profiles of herbicides used to control weeds in cotton.

The International Survey of Herbicide Resistant Weeds database (Heap, 2002) was searched for weed species with biotypes resistant to the major herbicides used to control weeds in cotton (identified in the Petition page 60 and 63). The total number of resistant species having resistance to either the same herbicide, or to another related member of the herbicide group to which it belongs, were included on **Table 1** below. Species with resistant populations that occur in cotton producing areas or states within the United States were listed, and these were checked to see whether they are listed as important weeds labeled for control by glufosinate-ammonium Liberty® herbicide in cotton (as indicated in the Petition, pg. 62). Liberty® herbicide was included in the search, but is not yet registered for use on cotton. Glyphosate, bromoxynil, and Liberty® are used in crop varieties resistant to the respective herbicide.

Appendix C. continued... Table 1.

Alternative Herbicides Used in Cotton	Herbicide Group/ HRAC Group	Mode of Action	Total # Resistant Weed Species	Resistant weed species that occur in cotton producing areas/states	Location of resistant biotypes	Target Weed of Liberty® in Cotton
trifluralin or pendamethalin	Dinitroanilines and others/K1	Microtubule assembly inhibition	10	Palmer amaranth	S. Carolina	Yes
				Goosegrass	S.E. USA, in cotton	No
				Annual bluegrass	N. Carolina	No
				Johnsongrass	MS	Yes
fluometuron	Ureas and amides/C2	PhotosystemII inhibitor	20	Barnyardgrass	AK, TX, MO, LA	Yes
MSMA	Organo-arsenicals/Z	Unknown	1	Common cocklebur	SE USA	Yes
pyrithiobac	Acetolactate Synthase (ALS) Inhibitor/B	ALS Inhibitor	73	Pigweed (3 *Amaranth* spp.)	SE, midwest USA	Yes
				Sunflower	MO, KS, SD, IA	Yes
				Perennial ryegrass	CA, TX, AR roadsides, wheat	No
				Italian ryegrass	MS roadsides	No
				Prickly sida	GA	Yes
				Johnsongrass	TX	Yes
				Common cocklebur	midwest U.S.	Yes
glyphosate (Roundup®)	Glycines/G	EPSPS inhibitor	4	None		No
bromoxynil	Nitriles and Others/C3	Photosystem II inhibitor	1	None		No
glufosinate-ammonium (Liberty®)	Glutamine synthase (GS) inhibitor/H	GS inhibitor	None			

Appendix D. Summary table of data submitted with the petition in support of nonregulated status for Aventis' LLCotton25.

Molecular Genetic Characterization and Stability
Vector map of plasmid pGSV71: Fig. III.1, page 22
Protein sequence of the PAT protein as produced in LLCotton25: Fig. III.2, page 23
Segregation analysis of transformation event LLCotton25: Table IV.1, page 25 and revised Table IV.1 in the Addendum
Schematic drawing showing the strategy for Southern hybridazation: Fig. IV.1, page 27
Summary of Southern hybridization data for the demonstration of the absence of vector sequences used for transformation: Table IV.3 , page 29
Southern Blot of LLCotton25 to demonstrate the absence of vector backbone, using Sm/Sp probe: Fig. IV.2, page 30
Southern Blot of LLCotton25 to demonstrate the absence of vector backbone, using 5'pVS1ori probe: Fig. IV.3, page 31
Southern Blot of LLCotton25 to demonstrate the absence of vector backbone, using 3'pVS1ori probe: Fig. IV.4, page 32
Southern Blot of LLCotton25 to demonstrate the absence of vector backbone, using ColE1 probe: Fig. IV.5, page 33
Demonstration of the insert stability in various generations: Figures IV.6 through IV.13, pp. 34-41
Phenotypic Characterization and Evidence Supporting Absence of Unintended Effects
Measurement of PAT protein levels in various raw agricultural commodities of LLCotton25: Table IV.4, page 44
Comparative analyses of agronomic characteristics between LLCotton25 and Coker312: Tables V.2 and V.3, pp. 49-50, and Appendices B and C, and of fruiting and reproductive characteristics, Appendices E and F.
Comparative analysis of composition of cottonseed and/or lint ,Tables V.4 - V.7, pp. 53-56.
Data on Environmental consequences of the introduction of LLCotton25
Summary of PAT toxicity to non-target organisms, pages 58 and 59
Potential impact on current agronomic practices, pages 60-61, and Aug. 9, 2002 Addendum
Comparison of Liberty Cotton system with herbicide regime, Table VI.2, page 63

Appendix E. Determination of Non-regulated Status for LLCotton25

In response to a petition (designated 02-042-01p) from Aventis CropScience USA LP, APHIS has determined that genetically engineered cotton transformation event LLCotton25 and progeny derived from it will no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. Permits or acknowledged notifications that were previously required for environmental release, importation, or interstate movement under those regulations will no longer be required for LLCotton25 cotton and its progeny. Importation of seeds and other propagative material would still be subject to APHIS foreign quarantine notices at 7 CFR Part 319 and the Federal Seed Act regulations at 7 CFR Part 201. This determination is based on APHIS' analysis of field, greenhouse, and laboratory data and references provided in the petition and other relevant information as described in this environmental assessment that indicate that LLCotton25 will not pose a plant pest risk for the following reasons. (1) It exhibits no plant pathogenic properties - although a plant pathogen was used in the development of this cotton, these plants are not infected by this organism, nor do they contain genetic material from this pathogen that can cause plant disease. (2) It exhibits no characteristics that would cause it to be more weedy than the non-transgenic parent cotton line or other cultivated cotton. (3) Gene introgression from LLCotton25 to native, introduced, or naturalized species of *Gossypium* in the United States is extremely unlikely, and it is not likely to increase the weediness potential of any resulting progeny nor adversely effect genetic diversity any more than would introgression from other cultivated cotton. (4) Disease and insect susceptibility and compositional profiles of the seeds and fiber of LLCotton25 are similar to those of its parent variety and other cotton cultivars grown in the United States, therefore, no direct or indirect plant pest effect on raw or processed plant commodities is expected. (5) Field observations, compositional analyses, and data on the safety of the engineered PAT protein all indicate that LLCotton25 should not have a greater potential than other cultivated cotton to damage or harm organisms beneficial to agriculture. (6) Compared to current cotton pest and weed management practices, cultivation of LLCotton25 should not reduce the ability to control pests and weeds in cotton or other crops. In addition to our finding of no plant pest risk, there will be no affect on threatened or endangered species resulting from a determination of non-regulated status for LLCotton25 and its progeny.

APHIS also has concluded that there may be new varieties bred from LLCotton25 cotton; however, they are unlikely to exhibit new plant pest properties, i.e., properties substantially different from any observed for cotton descended from LLCotton25, or those observed for other cotton varieties not considered regulated articles under 7 CFR Part 340.

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