USDA-APHIS Decision on Monsanto Petition 04-086-01p Seeking a Determination of Nonregulated Status for Glyphosate-Tolerant Cotton Line MON 88913

Environmental Assessment

TABLE OF CONTENTS

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 Roundup Ready® Flex cotton (*Gossypium hirsutum* L.) line MON 88913 (OECD Unique Identifier: MON 88913-8). MON 88913 has been genetically engineered for tolerance to the herbicide, glyphosate through the expression of an added gene derived from *Agrobacterium tumefaciens* strain CP4. APHIS received a petition (APHIS number 04-086-01p) from Monsanto Company (hereafter referred to as Monsanto) for a determination that MON 88913 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 Monsanto contains extensive information relevant to this determination. MON 88913 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 added gene were derived from plant pests and a plant pest was used as a vector for introduction of the added gene.

As a regulated article under the provisions of 7 CFR Part 340, the importation, interstate movement, or field tests of MON 88913 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.

In accordance with APHIS procedures for implementing the National Environmental Policy Act (NEPA) (7 CFR Part 372), this EA has been prepared prior to issuing a determination of nonregulated status for MON 88913 in order to specifically address the potential for impact to the human environment through the unconfined cultivation and use in agriculture of the regulated article.

II. BACKGROUND

A. Development of MON 88913

On July 11, 1995, APHIS approved a determination of nonregulated status for glyphosate tolerant cotton line 1445 (Petition No. 95-045-01p). Since then, this first-generation herbicide-tolerant cotton has been widely adopted by cotton farmers and has made up a significant portion of the U.S. cotton production. Line MON 88913 is a second-generation glyphosate tolerant cotton product that provides increased tolerance to glyphosate during the critical reproductive phases of growth compared to line 1445. Use of MON 88913 will enable the application of glyphosate herbicides over the top of the cotton crop at later stages of development than is possible with the line 1445. This will allow for effective weed control during crop production, because glyphosate is highly effective against the majority of annual and perennial weeds that can be problematic during the later stages of crop development, with minimal risk of crop injury. The increased level of glyphosate tolerance in MON 88913 is achieved through the use of

improved promoter sequences that regulate the expression of the *cp4 epsps* gene conferring glyphosate tolerance.

As in line 1445, Mon 88913 was developed by using recombinant DNA techniques to introduce a gene for EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), isolated from *Agrobacterium tumefaciens* strain CP4 that encodes an enzyme that confers tolerance to glyphosate, the active ingredient of Roundup® herbicide. As determined by Southern blot analysis, MON 88913 contains a single intact DNA insert from the binary plasmid PV-GHGT35 at a single integration locus within the cotton genome. While line 1445 contains one intact *cp4 epsps* gene expression cassette, the DNA insert in MON 88913 contains two intact *cp4 epsps* gene expression cassettes containing identical *cp4 epsps* coding sequences. Polymerase chain reaction was performed to confirm the 5' and 3' insert-to-genomic DNA junctions and the organization of elements within the insert in MON 88913. The DNA insert and the glyphosate tolerant trait are stable across multiple sexual generations. Phenotypic segregation data confirmed that the single insert locus and glyphosate tolerant trait behave as a single dominant locus with the expected Mendelian segregation pattern across multiple generations.

The CP4 EPSPS protein produced in MON 88913 is targeted to the chloroplasts via an N-terminal fusion with the chloroplast transit peptide, CTP2, to form a CTP2-CP4 ESPSP precursor protein. The precursor protein produced in the cytoplasm is processed to remove the transit peptide upon translocation into the plant chloroplast, resulting in the mature CP4 EPSPS protein. The transgenic cotton line that is the subject of the petition was developed by a widely used technique called Agro-infection which essentially involves using a plant pathogenic strain of *Agrobacterium tumefaciens* and its disarmed plasmid vector.

Roundup® herbicide contains the active ingredient glyphosate which is a non-selective, post-emergent weed control agent. The target site of action of glyphosate is EPSPS that is present in all plants, bacteria, and fungi as a component of the Shikimate pathway of aromatic amino acid biosynthesis (Levin and Sprinson, 1994). The CP4 EPSPS which results in increased resistance to glyphosate (Padgette et al. 1993) has been introduced into MON 88913 to confer tolerance to the foliar application of glyphosate.

Field tests of MON 88913 were conducted in more than 14 locations from 2000 to 2003 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. 163.) These locations provided a range of environmental and agronomic conditions representative of major U. S. cotton-growing regions where the majority of commercial production of MON 88913 is expected to occur. These tests were conducted, in part, to confirm that MON 88913 plants exhibit the desired agronomic and quality characteristics and do not pose a greater plant pest risk than the unmodified cotton. This was demonstrated by using a nontransgenic negative segregant of MON 88913 [MON 88913 (-)]. MON 88913 (-) has background genetics representative of MON 88913 but does not contain the *cp4 epsps* coding sequence or produce the CP4 EPSPS protein.

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 volunteer cotton plants.

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. MON 88913 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.). The Food and Drug Administration (FDA) enforces tolerances set by the EPA under the FFDCA. A submission of glyphosate residue data and proposed labeling for the expanded use of Roundup UltraMAX® herbicide (EPA Reg. No. 524-512) on Roundup Ready® Flex cotton, MON 88913, was made to the EPA on March 27, 2003. In the August 18, 2004 Federal Register, EPA published a notice of filing that they are in the process of developing residue tolerances.

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. In compliance with this policy, on May 28, 2004, Monsanto submitted to FDA a food and feed safety and nutritional assessment summary for Roundup

Ready® Flex cotton MON 88913 (BNF# 0098).

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 MON 88913 as a regulated article under APHIS regulations found at 7 CFR Part 340. The developer of MON 88913, Monsanto, submitted a petition requesting that APHIS make a determination that cotton transformation event MON 88913, and any progeny derived from crosses of event MON 88913 with other nonregulated cotton varieties, no longer be considered regulated articles under 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 MON 88913 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 MON 88913 cotton and its progeny.

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

Under this alternative, APHIS would reach a determination that MON 88913 and its progeny do not pose a plant pest risk and therefore, should no longer be considered regulated articles under 7 CFR Part 340. A basis for this determination would be established, which would result in a Finding of No Significant Impact (FONSI) under NEPA. 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 glyphosate herbicide on these plants by other regulatory agencies (e.g., registration with EPA).

C. Proposed Action: Determination of Nonregulaled 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." Two ways in which a petition might be approved in part are as follows:

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 MON 88913 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 MON 88913 or its progeny, cotton producers would still have the same options available to them for the control of weeds in cotton, including herbicides. However, it is important to note that since Roundup Ready® cotton (event 1445) was commercially planted in 1997, it has been adopted by many cotton farmers. The result has been a dramatic increase in the use of glyphosate herbicide and a similar decrease in the use of many other herbicides formerly used extensively in cotton (see Appendix E). Many of these are much more persistence in the environment and have the potential to carry over to the next crop. (Hager et al., 2000). It is expected that many growers will plant Roundup Ready® Flex cotton in place of Roundup Ready® cotton because it will give growers the flexibility of using glyphosate herbicide as an over the top herbicide application later in the growing season. Growers who currently plant Roundup Ready® cotton are restricted to other means of weed control once the plant grows a fifth true leaf. These methods include cultivation, use of glyphosate applied as a post-directed spray, and other herbicides. In 2002 an unpublished survey was conducted by Monsanto of over 500 U. S. cotton growers. Only 39% of the surveyed growers reported making a second over-the-top application of glyphosate, although permitted by the label. Although it is possible that some growers will make a second application later after the fifth true leaf has developed, the percentage is not likely to be much greater than the current number. It appears likely that the potential environmental impact from continued regulated status of MON 88913 would not be significant. Cotton farmers would continue to use existing technologies for the control of weeds.

The development of varieties based on MON 88913 and its progeny could increase weed control options to growers if the EPA also grants Monsanto's request to allow use of Roundup UltraMAX® herbicide on Roundup Ready® Flex cotton. However, granting nonregulated status does not guarantee the extent to which a new plant line, such as MON 88913, would be adopted by growers. As a regulated article, the field testing of MON 88913 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 MON 88913 without geographical restrictions. The APHIS assessment of environmental impacts of such a determination is discussed in the following sections. Environmental impacts of unrestricted cultivation of MON 88913 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 MON 88913 or in other plants, or to cause the production of plant pathogens. APHIS also considered whether data indicate that unanticipated plant pest effects would arise from cultivation of MON 88913. 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 from SEEDCO Corporation, Lubbock, 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 MON 88913 employed *Agrobacterium*-mediated transformation technology that utilized PV-GHGT35, a binary plasmid vector carrying the *cp4 epsps* gene construct within a disarmed transfer DNA (T-DNA) from *Agrobacterium tumefaciens* that lacks the phytohormone 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 MON 88913

The Monsanto petition provided data to support the conclusion that MON 88913 contains the T-DNA insert (petition, pages 39-55). The inserted DNA is comprised of two *cp4 epsps* gene expression cassettes of the T-DNA of plasmid PV-GHGT35, flanked by portions of the right and left border sequences derived from *Agrobacterium tumefaciens*: (1) the *ctp2/cp4 epsps* coding sequence whose transcription is directed by the chimeric promoter containing the *Arabidopsis thaliana tsfl* gene promoter, encoding elongation factor EF-1 alpha, and enhancer sequences from the figwort mosaic virus 35*S* promoter (FMV/TSF1), the leader (exon 1) and intron sequences from the *Arabidopsis thaliana tsfl* gene, and the transcriptional termination and polyadenylation sequence derived from the 3' nontranslated region of the pea (*Pisum* sativum) ribose-1, 5-biphosphate carboxylase (rbc) small subunit E9 gene; (2) a second *ctp2/cp4 epsps* coding sequence, identical to the first, whose transcription is directed by the chimeric promoter containing the promoter of the *act8* gene of *Arabidopsis thaliana* combined with the enhancer sequences of the cauliflower mosaic virus (CaMV), 35S promoter (35S/ACT8), the leader, intron and flanking sequences from the *act8* gene of *Arabidopsis thaliana,* and the transcriptional termination and polyadenylation sequence derived from the 3' nontranslational region of the pea (*Pisum sativum*) ribulose-1, 5-biphosphate carboxylase (*rbc*) small subunit E9 gene. This T-DNA was inserted into the cotton genome and results in the synthesis of a homogeneous CP4 EPSPS protein from the two *cp4 epsps* gene expression cassettes. The *ctp2* chloroplast transit peptide sequence, derived from the *Arabidopsis thaliana epsps* gene, is present to direct the CP4 EPSPS protein to the cotton chloroplast.

Of all of the DNA sequences inserted in the construction of MON 88913, only the 35S promoters are derived from organisms known to be plant pests (CaMV and FMV). Although CaMV and FMV are plant pathogens, the sequences included in MON 88913 cannot cause plant disease. They do not encode infectious entities and serve a purely regulatory function for the genes of interest. These sequences have a history of safe use in genetically engineered plants. 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 either of the 35S promoters cause plant disease or pose a plant pest risk in transgenic plants. Multiple generations of MON 88913 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 MON 88913 plants).

None of the other donor organisms used as sources for the DNA sequences engineered into the cotton to make MON 88913 are organisms with demonstrated plant pest characteristics.

Evaluation of intended effects in MON 88913:

As intended, MON 88913 expresses the 5-enolpyruvyshikimate-3-phosphate synthase (CP4 EPSPS) protein encoded by the *cp4 epsps* coding sequence. The Monsanto petition summarized

data which demonstrates the expression of this protein in plant tissues sufficient to confer the desired glyphosate resistance trait. Expression of the CP4 EPSPS protein was also detectable in transgenic seed and pollen. CP4 EPSPS protein content varied between different trial sites and between treatments with Roundup® (Page 64 of the petition). The Monsanto petition also summarized data which demonstrates that the glyphosate resistance trait conferred by this protein is inherited in a predictable manner when MON 88913 plants are crossed with other cotton plants (see petition pages 59-61 for Mendelian inheritance data). The petition provided data on field tests of MON 88913 in which the plants exhibited resistance to glyphosate. Treatments consisted of glyphosate sequentially applied over the top of MON 88913 and Roundup Ready® cotton at three different stages of growth. Glyphosate was applied using Roundup WeatherMAX® herbicide. The rate of glyphosate used at each application was 1.5 lb acid equivalent per acre (ae/A). Plants were sprayed initially with glyphosate at the approximately four-leaf (node) stage, and the second and third glyphosate applications were made when plants averaged 8 and 12 leaves (nodes), respectively. Thus, the plants received a total of approximately six times the recommended over-the-top single application rate of 0.76 lb ae/A. These field tests took place in 14 field trial locations conducted during 2002 to evaluate various parameters in addition to resistance to glyphosate, including emergence, seedling vigor, stand establishment and maturity (page 72 of the petition).

Evaluation of possible unintended effects in MON 88913:

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 MON 88913. Observations were made from seedling emergence through maturity on MON 88913 plants grown in 2002 in 14 field sites distributed in Mississippi, Arkansas, North Carolina, Texas, Tennessee, Missouri, Alabama, Georgia, Louisiana, South Carolina and Arizona (petition page 178). These states are among the top states in total cotton acreage planted in the United States (NASS, 2000). Fifth generation (R5) MON 88913 plants were compared to the nontransgenic negative segregant MON 88913 (-) that does not contain the *cp4 epsps* coding sequence or produce the CP4 EPSPS protein. MON 88913 (-) was used as a control rather than the parent variety Coker 312, because it is derived from MON 88913 and is expected to have background genetics which are more closely approximate. In addition to the field sites, the phenotypic evaluation is based on laboratory and greenhouse experiments. All studies were conducted by agronomists and scientists who are considered experts in the production and evaluation of cotton. Comparisons of phenotypic parameters between MON 88913 and MON 88913(-), and also to conventional cotton were conducted to establish the phenotypic and seed compositional equivalence of MON 88913. In evaluating the phenotypic characteristics of MON 88913, data were collected to assess the likelihood that the plant will harm the environment. These phenotypic characteristics have been grouped into five general categories: 1) dormancy, germination and emergence; 2) vegetative growth; 3) reproductive growth, maturity and overwintering capacity; 4) seed retention on plant; and 5) plant interactions with disease, insect, and abiotic stressors.

For the field sites, a randomized complete block design with four replications was employed for the comparisons and analysis. A total of 41 different phenotypic characteristics were evaluated including 11 characteristics during plant growth and development, 20 characteristics from plant mapping (height, nodes, height per node, etc.), four characteristics from boll/seed measurements, and six boll and fiber quality characteristics (petition pages 72-74, Table VII-2). In addition, observational data on the presence and any differential response to biotic (pests and disease) and abiotic stressors were collected. These measurements are well known to cotton researchers and can provide supplementary data to assess plant pest potential.

Out of a total of 458 comparisons between MON 88913 and MON 88913(-) by field location, 19 differences were detected at $p \leq 0.05$. There were no differences detected at any location between MON 88913 and MON 88913(-) for six of 11 plant growth and development characteristics measured, 13 of 20 plant map characteristics, two of four boll/seed measurements and three of six boll and fiber quality characteristics (petition, Appendix C). Most observed differences occurred for a single characteristic at a single field location. Furthermore, it is important to note that a frequency of differences of 4.15% (19/458 x 100) was less than the 5% level of error standard set for statistical significance, and further suggests that the transformation produced no significant impacts on the measured growth and development characteristics.

Seed dormancy is an important characteristic that is often associated with plants that are weeds (Anderson, 1996). Dormancy mechanisms, including hard seed, vary with species and tend to involve complex processes. Standardized germination assays of the Association of Official Seed Analysts (AOSA, 1998) are used as a baseline to measure the germination potential of cottonseed. Seed dormancy characteristics were compared between MON 89913, MON 88913(-), and six conventional cotton varieties to assess the potential impact of the presence of the DNA insert or the CP4 EPSPS protein produced in MON 88913 on cottonseed dormancy (petition Appendix C, Tables C-2,3,4). The tested seed were produced during 2002 at three field locations within the U.S. cottonbelt: Baldwin County, AL; Tulare County, CA; and Clarke County, GA, representing environmentally relevant conditions for cotton production. Out of 87 comparisons between MON 88913 and MON 88913(-), 75 were not statistically significant at $p \leq 0.05$. No differences between MON 88913 and MON 88913(-) were detected for seed dormancy-related characteristics, such as hard seed, with seed from any location. Of the significant differences detected five were a result of reduced germination for MON 88913 compared to MON 88913(-), with another five differences resulting from the same plants showing a rise in dead seed. Decreased germination accompanied by more dead seed with no changes in hard or viable firm swollen seed would not indicate increased weed potential of MON 88913. The remaining two statistical differences were detected between MON 88913 and MON 88913(-) in the 10/20°C temperature regime for percent dead seed (CA location) and percent viable firm swollen seed (GA location). These differences were very small and values from MON 88913 were within the ranges observed from conventional cotton produced at each respective location.

The lack of meaningful differences between MON 88913, MON 88913(-), and conventional cotton varieties indicate that the presence of the DNA insert or the presence of the CP4 EPSPS protein did not alter the seed dormancy and germination characteristics of MON 88913. These data suggest that there was no change in the weed potential of MON 88913 as a result of increased dormancy or from changes in germination characteristics, further supporting phenotypic equivalence and familiarity.

Expression of the CP4 EPSPS protein is not expected to cause plant disease or influence the susceptibility of MON 88913 to plant pathogens or pests. Monsanto evaluated the expression levels of this protein in MON 88913 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 MON 88913 plants compared to Mon 88913 (-) control cotton plants that had no CP4 EPSPS gene. Out of seven disease and 38 abiotic stressor observations, no differences were detected between MON 88913 and MON 88913(-) (petition Appendix C, Table C-10). These results support the conclusion that environmental interactions of MON 88913 are not expected to be different than that of other cotton.

Compositional analysis is useful to indicate whether levels of nutrients, antinutrients, toxicants or other components of MON 88913 are altered relative to the appropriate control and to commercial conventional cotton. Analysis were conducted on the cottonseed to measure proximate composition (protein, total fat, ash, and moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), crude fiber, total dietary fiber (TDF), amino acids, fatty acids (C8-C22), cyclopropenoid fatty acids (malvalic acid, sterculic acid, and dihydrosterculic acid), vitamin E, minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), gossypol (free and total), and aflatoxins (B1, B2, G1, and G2). In addition, carbohydrates and calories were determined by calculation. The results demonstrate that the levels of key nutrients and other components of cottonseed of MON 88913 are within the expected range for conventional cotton (petition, Appendix E).

The overall conclusions from this extensive phenotypic characterization were that there are no biologically meaningful differences in terms of pest potential between MON 88913 and MON 88913(-) and the phenotype of cotton has been changed only with respect to the Roundup Ready trait.

These observations provide further evidence that MON 88913 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 MON 88913 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 MON 88913 compared to currently cultivated cotton varieties.

APHIS evaluated whether MON 88913 would be any more likely to become a weed than the

negative segregant MON 88913(-), the parental line Coker 312, or 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 Monsanto petition that substantiated that MON 88913 derived lines were similar to nontransgenic counterpart varieties when grown over a variety of locations, with or without glyphosate 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. When all data were pooled across locations, a single statistically significant difference in the growth and development characteristics was observed. The date until 50% flowering was later for MON 88913 compared to MON 88913(-) (64 vs. 63 days after planting, respectively) (petition page 75, Table VII-3). This difference was one day at most sites, has little biological meaning in terms of plant weed potential, and could be because of small differences in the background genetics between MON 88913 and MON 88913(-), due to an inherent natural variability within cottonseed. The genetic background of MON 88913(-) is expected to be very close, but not 100% identical, to that of MON 88913 (see petition addendum, page 4). Some locations showed a reduced germination rate and an increase in dead seed of MON 88913 when compared with MON 88913(-). Decreased germination accompanied by more dead seed with no changes in hard or viable firm swollen seed would not indicate increased weed potential of MON 88913.

In addition to the results summarized above, APHIS notes that there have been no reports of increased weediness associated with the commercial plant that is most similar to MON 88913, namely its parent Coker 312. On the basis of all the submitted data and field observations to date, MON 88913 appears to pose no greater plant pest risk of weediness than that posed by traditional cotton cultivars.

3. Potential impacts from gene introgression from MON 88913 to its sexually compatible relatives.

MON 88913, 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). APHIS considered whether such crosses are likely to occur when MON 88913 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. MON 88913 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* or *G. tomentosum* are listed as weeds, on either 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. *G. hirsutum* tends to be pollinated by bumblebees during the day. It has been thought that *G. tomentosum* was pollinated by moths at night. Recent preliminary results of EPA funded research by Drs. John Pleasants and Jonathon Wendel of Iowa State University on *G. tomentosum* populations in Hawaii provide new information about their distribution, the timing of flowering, and potential pollinators (Memorandum dated April 8, 2004 to Janet Andersen, Director, Biopesticides and Pollution Prevention Division (BPPD), USEPA, from Tessa Milofsky, Regulatory Action Leader, BPPD, documenting a conversation with Jonathon Wendell regarding their research; and personal communication from Dr. Pleasant to Susan Koehler, USDA, APHIS, June 1, 2004). Natural populations are found on all the islands except Kauai (in contrast to historical records) and Hawaii. The species is dominant on the Hawaiian island of Kohoolawe and several sizable populations were found on the islands of Oahu and Maui. The sparse populations observed on Molokai appear to be threatened by recent ecological alterations, resulting from farming and ranching activites that have decimated much of the island's native flora. In some places *G. tomentosum* has been planted for habitat restoration or roadside or stream bank stabilization. Dr. Pleasants indicated that there was no evidence that it is being controlled as a weed in any of the habitats that they have observed. While the plants are primarily self-pollinating, in contrast to earlier reports that the flowers open at night and may be cross-pollinated by a moth, their research found that the flowers appear to open at sunrise, and pollen is viable until about 4-5 pm, corresponding with the pollination window for *G. hirsutum*. Furthermore, hymenopteran insects, including the honey bee, carpenter bee, and an unidentified small black bee, were observed as frequent visitors and possible pollinators in *G. tomentosum.* Although bees are capable of transporting pollen long distances (up to 12 km from their hive), the researchers noted that the

homogeneity of the *G. tomentosum* populations suggests that insect mediated pollination events are infrequent between distant populations.

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 Monsanto petition that substantiates that no consistent significant differences were observed between MON 88913, the negative segregant MON 88913(-), 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 C, pp 171 - 202). Therefore, there is no reason to suspect that MON 88913 would have a greater outcrossing rate.

In sum, APHIS believes that it is very unlikely that MON 88913 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 MON 88913 (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 *cp4 epsps* gene will introgress from MON 88913 into *G. thurberi* and *G. tomentosum*. In the registration requirements for the early 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 MON 88913 to wild cotton relatives is not likely, and if it occurs, would not lead 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 MON 88913 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. The CP4 EPSPS protein contained in MON 88913 and other Roundup Ready® crops are similar to the native EPSPS protein that is ubiquitous in plant and microbial tissues in the environment (Petition, Appendix D, pages 204-211). Therefore, based on this history of occurrence, the EPSPS protein is not expected to possess biological activity towards nontarget organisms. Even though the likelihood of hazard is low for the CP4 EPSPS protein, a number of researchers have conducted laboratory investigations with different types of arthropods exposed to Roundup Ready® crops containing the CP4 EPSPS protein (Goldstein, 2003; Boongird et al., 2003; Jamornman, et al., 2003; Harvey et al., 2003). Representative pollinators, soil organisms, beneficial arthropods and pest species were exposed to tissues (pollen, seed, and foliage) from Roundup Ready® crops that contain the CP4 EPSPS protein. These studies, although varying in design, all reported a lack of toxicity observed in various species exposed to Roundup Ready® crops producing the CP4 EPSPS protein (Nahas et al., 2001; Dunfield and Germida, 2003, Siciliano and Germida 1999). It is expected that the CP4 EPSPS protein in cotton would have a similar lack of effect as CP4 EPSPS in other crop plants.

The lack of toxicity is further supported by field experimentation conducted on biotechnology-derived crops producing the CP4 EPSPS protein. Diversity and abundance of Collembola was no different between Roundup Ready soybeans and conventional soybeans grown under the same management systems (Bitzer et al., 2002). Other studies on registered Roundup Ready® soybeans under various weed management systems concluded that there was no apparent direct effect of the Roundup Ready® trait on arthropods, although weed management and phenotypic differences (plant height or maturity) associated with plant variety influenced arthropod populations (Jasinski et al., 2003; McPherson et al., 2003; Buckelew et al., 2000). A similar lack of effect on arthropods seen in soybeans is expected for MON 88913 cotton.

In addition to the lack of observed toxicity of the CP4 EPSPS protein, the compositional analysis of MON 88913 (Section VII.C; Appendix E), found that there were no significant differences between MON 88913 and MON 88913(-) for the toxicants (aflatoxins B1, B2, G1, and G2), gossypol (free and total), and there were no significant differences in the combined site analysis for the antinutrient cyclopropenoid fatty acids (malvalic acid, sterculic acid, and dihydrosterculic acid). A significant difference in malvalic acid and sterculic acid occurred at a single site, which did not occur at the other three locations. These observed differences are unlikely to be biologically meaningful because the range of values for these analytes were found to fall within the 99% tolerance interval for the commercial varieties planted in the same field trials as MON

88913 and MON 88913(-). Therefore there is no reason to anticipate that MON 88913 would impact nontarget organisms beyond that expected for other cotton plants.

APHIS has never encountered impacts on nontarget organisms associated with the expression of CP4 EPSPS. Cotton (*G. hirsutum*) is not sexually compatible with any plant species listed as threatened or endangered. The genetic modification in MON 88913 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 glyphosate-tolerant cotton MON 88913 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 Roundup UltraMAX® herbicide on Roundup Ready® Flex 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 labeling request.

5. Potential Impacts on Biodiversity

After careful evaluation, APHIS believes that MON 88913 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 nontarget 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 MON 88913 will pose a significant impact on biodiversity.

6. Potential Impacts on Agricultural and Cultivation Practices

APHIS considered the potential impacts of MON 88913 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 MON 88913 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. 112-119 and July 9, 2004 Addendum Page 7) 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 MON 88913 and the potential expanded registration of the Roundup UltraMAX® herbicide for use on Roundup Ready® Flex cotton.

A variety of herbicides and cultivation practices are recommended for weed control in cotton

(Vargas et al., 2001). Recently cotton varieties tolerant to herbicides (those resistant to the broadleaf herbicide bromoxynil or to the broadspectrum herbicides glyphosate and glufosinate) have been grown over fairly large acreage 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. 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), although between 1997 and 2003 the area of cotton treated with pyrithiobac has declined by 25.9%. For other herbicides used on cotton during this period, moderate decreases in use have been reported for metolachlor (-17.8%), trifluralin (-22.2%), pendimethalin (-25.6%), and prometryn (-28.1%). More significant reductions in use have been observed for fluometuron (-84.1%), MSMA/DSMA (-78.6%), cyanazine (-97.6%), norflurazon (-97.1%), clomazone (-96.7%) and the post-emergence graminicides (clethodim, fluazifop-p-butyl, quizalofop-ethyl, and sethoxydim) (-87.8%). Since its introduction into cotton in combination with BXN® cotton, bromoxynil use has also declined by 97.3% since its peak in 1999 with a corresponding decrease in the planting of BXN® cotton. Glyphosate use in cotton has increased since the introduction of Roundup Ready® cotton in 1997, with a corresponding increase in reduced- and no-till cotton practices in cotton. Other than glyphosate, diuron is the only other cotton herbicide with increased usage since 1997. This is likely the result of the voluntary withdrawal of the cyanazine (Bladex®) use label in cotton in the late 1990s. (July 9, 2004 Petition Addendum, page 6 – see Appendix E for more details). In 2002 Roundup Ready® cotton was planted on approximately 59% of U.S. cotton acreage (USDA-NASS 2003b). This switch to glyphosate from several other herbicides is seen as a positive occurrence. Glyphosate is much less persistent in the environment and is less toxic than most of the herbicides that had a reduced use on cotton (Hager et al., 2000, and Ferrel et al., 2004).

Glyphosate is currently registered under various trade names for control of weeds in other crops, and as Roundup® for use on Roundup Ready® cotton, corn, canola, and soybean varieties. In conventional plants, glyphosate binds to the endogenous plant EPSPS enzyme and blocks the biosynthesis of EPSP, thereby depriving plants of essential amino acids (Steinrucken and Amrhein, 1980; Haslam, 1993). In Roundup Ready® plants, which are tolerant to glyphosate agricultural herbicides, aromatic amino acids and other metabolites that are necessary for growth and development are met by the continued action of the CP4 EPSPS enzyme in the presence of glyphosate (Padgette et al., 1993). MON 88913 contains the CP4 EPSPS gene and therefore growers will likely use glyphosate for weed control.

Today, some 171 herbicide-resistant species and 286 biotypes within those species have been identified (Heap, 2004). Most of them are resistant to the triazine family of herbicides (Holt and Le Baron, 1990; Le Baron, 1991; Shaner, 1995). Resistance usually has developed because of the long residual activity of these herbicides with the capacity to control weeds all year long and the selection pressure exerted by the repeated use of herbicides with a single target site and a specific mode of action. Using these criteria, and based on current use data, glyphosate is

considered to be a herbicide with a low risk for weed resistance (Benbrook, 1991). To date, biotypes of only four weed species resistant to glyphosate have been identified and confirmed. In all cases, Monsanto worked with local scientists to identify alternative control options that have been effective in managing the resistant biotypes. These include annual ryegrass (*Lolium rigidum*) in Australia, California and South Africa; Italian ryegrass (*Lolium multiflorum*) in Chile and Brazil; goosegrass (*Eleusine indica*) in Malaysia; and marestail (*Conysa canadensis*) in certain states of the eastern and southern U.S. (Petition, Appendix F, page 230). Of the four, only *Conysa canadensis* has been observed in cotton fields. The number of sites and acres in this area of the country is on the increase (Heap, 2004). However, it is uncertain if the use of Round Ready® cotton has contributed to its spread. The mechanism of resistance in this biotype is currently under investigation by Monsanto. Their current working hypothesis is that marestail resistance results from an alteration of glyphosate distribution that impairs its phloem loading and plastidic import (Petition, Appendix F, page 231).

If the Roundup UltraMAX® registration is expanded to include use on Roundup Ready® Flex cotton, then the possible commercial use of varieties based upon MON 88913 may have positive impacts on current agricultural practices. It could provide an opportunity to use Roundup UltraMAX® as an alternative broad-spectrum, post-emergent herbicide in cotton with a wider 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. Volunteers of cotton are not normally a problem but if volunteers of MON 88913 based varieties were to occur, they could potentially be controlled by a number of herbicides used in crops grown in rotation with cotton (Petition, page 121). 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 glyphosate resistant.

Several transgenic cotton varieties that are either insect or herbicide resistant are already in widespread use by farmers. Varieties derived from MON 88913 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 MON 88913 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 *cp4 epsps* gene and its CP4 EPSPS protein, support the safety of MON 88913 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 Roundup UltraMAX® herbicide registration be expanded to include use on Roundup Ready® Flex cotton, cultivation of glyphosate-tolerant cotton varieties derived from MON 88913 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 glyphosate. The use of herbicides in cotton cultivation is regulated by the EPA. Tolerance levels are established by taking into account the cumulative exposure of 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 MON 88913 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 Cotton Event MON 88913. It should 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. Any international traffic in cotton subsequent to these determinations 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 and transboundary movement of particular agricultural commodities produced through biotechnology are being addressed in international forums and through national regulations. The Cartegena Protocol on Biosafety is a treaty under the Convention on Biological Diversity that established a framework for the safe transboundary movement, with respect to the environment and biodiversity, of living modified organisms (LMOs), which includes those modified through biotechnology. The protocol came into force on September 11, 2003 and 82 countries are parties to it as of Jan. 21, 2004 (see http://www.biodiv.org/biosafety/default.aspx.). Although the United States is not a party to the CBD, and thus not a party to the Cartegena Protocol on Biosafety, US exporters will

still need to comply with domestic regulation that importing countries that are parties to the Protocol have put in place to comply with their obligations. The first intentional transboundary movement of LMOs will require consent from the importing country under an advanced informed agreement (AIA) provision and the required documentation. To facilitate compliance with obligations to this protocol, the US Government is developing a website that provides the status of all regulatory reviews completed for different uses of the product. This data will be available to the Biosafety Clearinghouse database that contains regulatory decisions for LMOs that may be subject to the Biosafety Protocol.

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, and NAPPO has developed a standard for the *Importation and Release into the Environment of Transgenic Plants in NAPPO Member Countries* (see http://www.nappo.org/Standards/Std-e.html.). 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 discussions on biotechnology regulatory issues with other countries (e.g. with Canada, Mexico, Argentina, Brazil, Japan, China, Korea to name a few), 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 and both countries have ratified the Cartegena Protocol. APHIS does not expect a significant environmental impact outside the United States should nonregulated status be granted for the subject Cotton Event MON 88913.

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 MON 88913 and any progeny lines derived from it by traditional breeding practices. Therefore, APHIS can consider only MON 88913 for approval.

2. Approval of the petition with geographic restrictions.

EPA is currently reviewing the petition to include the use of Roundup UltraMAX® on Roundup Ready® Flex cotton. 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 nontarget 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 MON 88913 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 MON 88913 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 has 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.

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 MON 88913 conducted under APHIS authorizations.

Table B. 1.

USDA Reference Number	Effective Date	Approved Release Sites (by state) Covered by Notification
2000 Field Trials:		
00-140-06n	6/22/00	PR
00-213-01n	9/11/00	PR
2001 Field Trials:		
00-362-01n	1/29/01	AZ, TX
01-031-02n	3/22/01	AL, AR, AZ, GA, MS, NC, SC, TN, TX
01-058-07n	3/29/01	IL
01-232-02n	9/20/01	PR
2002 Field Trials:		
02-004-11n	2/3/02	TX
02-016-27n	2/15/02	LA
02-018-16n	2/17/02	AZ
02-022-50n	2/21/02	CA
02-022-54n	3/26/02	AL
02-022-55n	2/21/02	MO
02-023-15n	3/20/02	TN
02-023-16n	2/27/02	AL, AR, LA, MS, NC, SC, TX
02-025-01n	2/24/02	NC, SC
02-025-02n	2/24/02	MS
02-025-07n	2/24/02	GA
02-025-08n	2/24/02	TX
02-025-09n	2/24/02	IL
02-028-28n	2/27/02	AR
02-042-31n	3/13/02	AL, CA, GA, TX
02-044-12n	3/15/02	AR, AZ, GA
02-046-12n	3/17/02	AR, GA, MS, OK
02-046-14n	3/17/02	TX
02-046-15n	3/17/02	AZ
02-051-22n	3/22/02	CA
02-221-08n	9/11/02	PR

Table B-1 (Continued). MON 88913 Field Trials.

USDA Reference Number	Effective Date	Approved Release Sites (by state) Covered by Notification
2003 Field Trials:		
02-282-09n	11/21/02	AZ, MS, TX
03-022-06n	2/21/03	TX
03-023-03n	2/22/03	TN
03-027-01n	2/26/03	AL, GA, MS, NC
03-027-03n	2/26/03	TN
03-030-05n	3/31/03	AL, AR, AZ, FL, GA, LA, MO, MS, NC, OK, SC, TX
03-030-12n	3/1/03	CA
03-038-02n	3/9/03	AZ, MS, TN
03-042-10n	3/13/03	AZ
03-042-11n	3/13/03	AL
03-042-12n	4/4/03	GA
03-042-13n	3/13/03	MS
03-042-14n	3/13/03	TX
03-042-19n	3/13/03	AZ, CA
03-043-13n	3/14/03	OK
03-052-23n	3/23/03	TX
03-052-29n	3/23/03	AR, CA, MS
03-052-45n	3/23/03	TX
03-052-46n	3/23/03	TX
03-052-47n	3/23/03	AZ, MS
03-059-03n	3/30/03	MS, SC
03-071-04n	4/11/03	AR
03-100-03n	5/10/03	IL
03-112-11n	5/22/03	GA
03-115-04n	5/25/03	AR
03-224-02n	9/11/03	PR
03-226-04n	9/23/03	PR
03-226-05n	9/23/03	PR
03-226-06n	9/23/03	PR
03-226-07n	9/13/03	PR
03-226-08n	9/13/03	PR
03-226-09n	9/13/03	PR
03-226-10n	9/13/03	PR
03-227-01n	9/23/03	PR
03-227-02n	9/14/03	PR
03-317-01n	12/13/03	AR, TX

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. 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 glyphosate herbicide in cotton. Glyphosate, bromoxynil, and glufosinate-ammonium are used in crop varieties resistant to the respective herbicide.

Table C. 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 Roundup Ultra-MAX® in Cotton
trifluralin or Pendimethalin	Dinitroanilines and others/K1	Microtubule assembly inhibition	10	Palmer amaranth	S. Carolina	Yes
				Goosegrass	S.E. USA, in cotton	Yes
				Annual bluegrass	N. Carolina	Yes
				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	Yes
				Italian ryegrass	MS roadsides	Yes
				Prickly sida	GA	Yes
				Johnsongrass	TX	Yes
				Common cocklebur	midwest U.S.	Yes
glyphosate (Roundup®)	Glycines/G	EPSPS inhibitor	4	Marestail	TN. AR, MS, LA	Yes*
Bromoxynil	Nitriles and Others/C3	Photosystem II inhibitor	1	None		No
glufosinate-ammonium (Liberty®)	Glutamine synthase (GS) inhibitor/H	GS inhibitor	None			

* Has supplemental label for glyphosate resistant marestail.

Appendix D. Summary table of data submitted with the petition in support of nonregulated status for MON 88913.

List of Tables

Appendix E. Changes in Herbicide Use in Cotton Since 1997

Herbicide	2003		1997		% Change[b]
	Area Treated %	Total Applied[a]	Area Treated %	Total Applied[a]	
Glyphosate	70	13,637	14	1,599	752.9
Trifluralin	39	4,404	55	5,663	-22.2
Diuron	28	1,842	12	916	101.1
Pendimethalin	20	1,921	28	2,583	-25.6
Pyrithiobac-sodium	12	131.4	23	177.3	-25.9
Prometryn	11	1,245	19	1,731	-28.1
Fluometuron	8	800	44	5,026	-84.1
MSMA/DSMA	7	1,245	33	5,817	-78.6
Metolachlor	5	626[c]	5	762	-17.8
Cyanazine	<0.5	55.1	18	2,283	-97.6
Norflurazon	<0.5	30.7	13	1,077	-97.1
Clomazone	<0.5	17.0	8	518	-96.7
Bromoxynil	<0.5	16.0	7[d]	593[d]	-97.3
Graminicides[e]	<0.5	14.8	7	121	-87.8
States surveyed[1,2]	AL, AZ, AR, CA, GA, LA, MS, MO, NC, SC, TN, TX		AL, AZ, AR, CA, GA, LA, MS, MO, NC, SC, TN, TX		
Acreage represented[1,2]	12,795,000		13,075,000		
Total planted cotton acreage[3,4]	13,301,000		13,558,000		

[a] 1000 lbs. Calculated values adjusted to reflect total upland cotton acreage planted for respective years.

[b] Percent change to total applied (lbs.). Calculated values adjusted to reflect total upland cotton acreage planted. Values normalized to 1997 upland cotton planted acreage.

[c] Includes both racemic and S-forms of metolachlor.

[d] Bromoxynil calculated values based upon 1999 upland cotton planted acreage[5,6].

[e] Clethodim, fluazifop-p-butyl, quizalofop-ethyl, and sethoxydim

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